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Chemical Behavior of Systox Isomers in Biological Systems[1,2]

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University of California Citrus Experiment Station, Riverside

A thorough knowledge of the chemical behavior and reactions of systemic insecticides in biological systems is essential in order to define clearly the nature of the toxic substance or substances derived by the system. Systemic insecticides are defined as those which are absorbed and translocated to other parts of the plant, thus exerting their toxic influence on insects throughout the entire plant. A systemically active substance may exert its action by being absorbed and distributed unchanged, as appears to be the case with some derivatives of 2-fluoroethyl alcohol (Schrader 1952), or it may be acted upon by certain systems in the plant and converted to toxic metabolic products. In general, the organo-phosphorus compounds appear to fall in the latter category, and it is with these substances that a great deal of effort has been expended upon the elucidation of their chemical behavior in biological systems. The identification and knowledge of the physico-chemical properties of these metabolic poisons is important not only from the standpoint of residual hazards to mammals but also for a clearer understanding of the systems involved, thus laying the foundation for the development of future systemics.

The active ingredients in commercial Systox,[3] which is insecticidally active both systemically and by contact, consist of the pair of structural isomers, *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate (thiono-phosphate) and *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate (thiolphosphate). The chemical and physical properties of these isomers have been described in detail and they appear to differ widely (Gardner & Heath 1953, Fukuto & Metcalf 1954, Heath *et al.* 1953). The systemic action of Systox, however, is not due to the action of the unchanged isomers, but to some metabolic product or products which are toxic to mammals and insects. It has been shown by Heath *et al.* (1953) that when the roots or foliage of brassicaceous plants are treated with the thiono- or thiolphosphate, essentially none of the isomers can be found in the plant after a few days. Heath believes that at least three different nonionic metabolic products are obtained from each of the isomers. By a solvent partition procedure, three metabolites, D1, D2, and D3 were isolated from the thiol isomer. These substances were not identified, but their properties were studied.

From numerous investigations concerning the fate of octamethylpyrophosphoramide (schradan) in plants and in mammals it is believed that schradan is converted to the toxic amine oxide and methylol derivatives by an oxidative process (Kilby 1953). Both the thiono- and the thiolphosphate contain functional groups which are highly susceptible to oxidation. The information presented here indicates that the metabolic products of the isomers are identical with certain oxidative products.

Materials and Methods.—*Tracer Preparation.*—P^{32}-labeled *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate and *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate were prepared as described previously (Fukuto & Metcalf 1954), starting with P^{32}-phosphorus trichloride (10 mc., obtained from Tracerlab Inc., Boston, Mass.). From 4.67 g. of phosphorus trichloride was obtained 3.5 g. of thiono isomer with an initial activity of 2.4 c.p.s./γ. Chromatographic analysis showed that the radioactive constituent was 96.5 per cent thiono isomer. A 1-g. sample of this material was converted to an 86 per cent thiol isomer by heating at 120° C. for 4 hours.

Hydrogen Perioxide Oxidations and the Preparation of The Derivatives of the Systox Isomers.—To three different samples consisting of 0.1 g. of the thionophosphate dissolved in a small amount of acetone was added 0.2 g. of 30 per cent hydrogen peroxide at room temperature. The mixtures were allowed to stand for 1 hour, 18 hours, and 3 days respectively. Water was then added and the products were extracted with 3 10-ml. portions of chloroform. After washing with 2 20-ml. portions of 10 per cent aqueous ferrous sulfate and water, the chloroform solutions were dried over anhydrous sodium sulfate and concentrated under water aspirator pressure. Chromatographic analysis on propylene glycol paper showed that the thionophosphate had been converted to at least two different oxidative products (see Fig. 1). In the 3 days' oxidation mixture, elemental sulfur also separated from the solution; this indicates the conversion of the thiono (P=S) to a phosphate moiety (P=O). The oxidation of the thiolphosphate was carried out in the same manner and at least one oxidative product was found by chromatographic analysis.

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[2] Part II of a series of papers on the mode of action of Systox. This work was supported in part by grants from the U. S. Atomic Energy Commission, Contract AT (11-1) 34, Project 6, and from the Chemagro Corporation, New York, N. Y.
[3] Trade-mark, Chemagro Corporation, New York, N. Y.

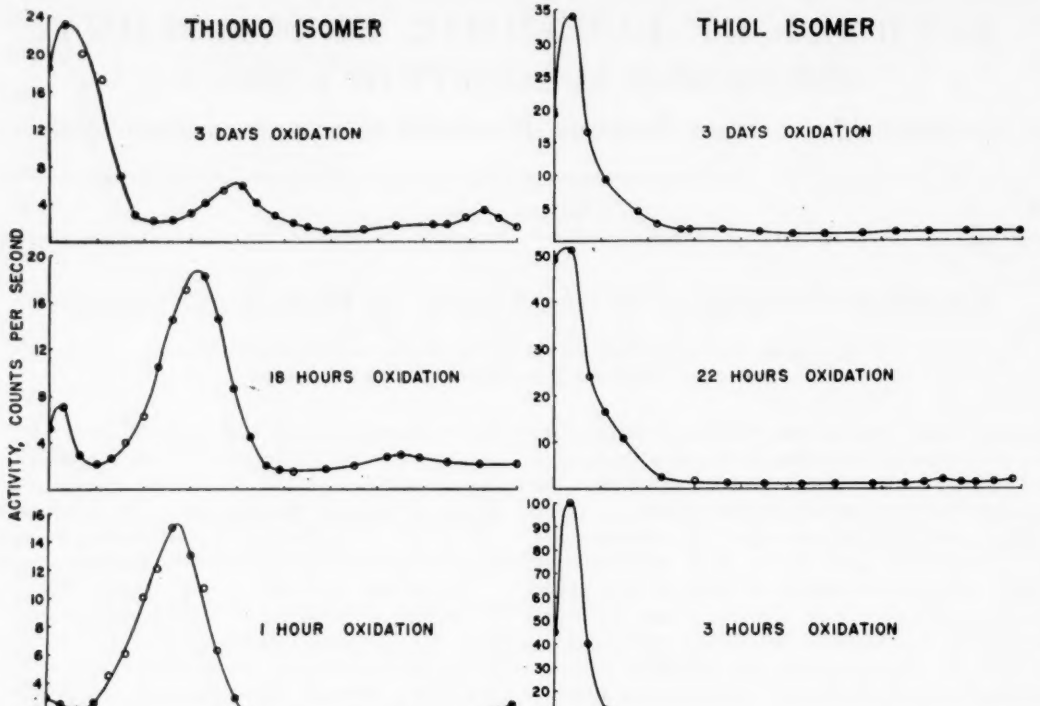


FIG. 1.—Propylene glycol paper chromatograms of P^{32}-labeled thiono and thiol isomers after oxidation with hydrogen peroxide for varying intervals.

O,O-Diethyl O-ethyl-2-sulfinylethyl phosphorothionate (*thionophosphate sulfoxide*).—To 25 g. of *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate in 25 ml. acetone, was added, with stirring, 15 g. of 30 per cent hydrogen peroxide. After the mixture had been stirred for 4 hours at room temperature, the solution was poured into water, and the oil which separated was taken up on chloroform. The chloroform solution was washed with aqueous ferrous sulfate, water and dried over anhydrous sodium sulfate. The chloroform was removed under vacuum and the product was distilled in the falling-film molecular still. The main fraction, which amounted to 10 g., was collected at 160° C. (0.3 mm.), n_D^{26} 1.4930. Elemental analysis: per cent calculated for $C_8H_{19}S_2O_4P$, C = 35.0, H = 6.98; found, C = 34.32, H = 6.55.

O,O-Diethyl S-ethyl-2-sulfinylethyl phosphorothiolate (*thiolphosphate sulfoxide*).—The thiolphosphate sulfoxide was prepared by stirring for 3 hours 25 g. of *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate and 15 ml. of 30 per cent hydrogen peroxide in 25 ml. acetone. The product was isolated in the usual manner and distilled in the falling-film still, b.p. 160° C. (0.3 mm.), n_D^{26} 1.5016. Elemental analysis: per cent calculated for $C_8H_{19}S_2O_4P$, C = 35.0, H = 6.98; found, C = 35.12, H = 7.12.

O,O-Diethyl O-ethyl-2-mercaptoethyl phosphate (*phosphate*).—To a mixture of 7 g. of sodium hydride and 50 ml. of anhydrous toluene was added slowly, with stirring, 30.6 g. of 2-ethylmercaptoethanol. The mixture was then stirred and heated at reflux until the sodium hydride disappeared. The mixture was allowed to cool and 50 g. of diethoxyphosphoryl chloride was added dropwise. After it had been stirred for 1 hour the toluene solution was washed with water, treated with Nuchar, and dried over sodium sulfate. The solvent was removed under vacuum and the product was distilled, b.p. 115–116° C. (0.3 mm.), n_D^{23} 1.4516. The yield was 35 g. Elemental analysis: per cent calculated for $C_8H_{19}PO_4S$, C = 39.65, H = 7.90; found, C = 40.07, H = 7.82.

O,O-Diethyl O-ethyl-2-sulfinylethyl phosphate (*phosphate sulfoxide*).—This compound was prepared in the same manner as the other sulfoxides from the above phosphate and hydrogen peroxide. The product distilled at 170° C. (1 mm.), n_D^{24} 1.4597. Elemental analysis: per cent calculated for $C_8H_{19}PO_5S$, C = 37.20, H = 7.42; found, C = 37.51, H = 7.50.

O,O-Diethyl O-ethyl-2-sulfonylethyl phosphate (*phosphate sulfone*).—To a mixture of 5 g. 2-hydroxydiethyl sulfone, 15 ml. anhydrous carbon tetrachloride, and 4 ml. anhydrous pyridine, 6.25 g. of diethoxyphosphoryl chloride was added dropwise at room temperature. The mixture was stirred for 4 hours, after which the solution was washed with dilute hydrochloric acid, aqueous sodium carbonate, and water and was dried over anhydrous sodium sulfate. The solvent was removed and the product was distilled under reduced pressure, b.p. 155–159° C. (0.3 mm.), n_D^{28} 1.4490. The yield was 2 g. Elemental analysis: per cent calculated for $C_8H_{19}O_6SP$, P = 11.3; found, P = 11.5.

O,O-Diethyl S-ethyl-2-sulfonylethyl phosphorothiolate (thiolphosphate sulfone).—To 8 g. of *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate in 10 ml. acetic acid was added 6 g. potassium permanganate dissolved in 130 ml. water. The mixture was stirred for 2 hours, decolorized by the addition of sodium bisulfite and extracted three times with chloroform. The chloroform solution was dried over anhydrous sodium sulfate and the product was distilled in the falling-film molecular still. The main fraction, which amounted to 5 g., was collected at 160° C. (0.2 mm.), n_D^{26} 1.4909. Elemental analysis: per cent calculated for $C_8H_{19}S_2O_5P$, P = 10.7; found, P = 10.9.

2-Hydroxydiethyl sulfone.—To 50 g. of 2-hydroxydiethyl sulfide in 50 ml. acetic acid was added 55 ml. of 30 per cent hydrogen peroxide. The mixture was allowed to stand overnight and another 80 ml. of hydrogen peroxide was added. The clear solution was then heated for 2 hours on the steam bath and the product was distilled, b.p. 129–130° C. (0.3 mm.). The 2-hydroxydiethyl sulfone crystallized upon standing, m. p. 43–45° C.

O,O-Diethyl O-ethyl-2-sulfonylethyl phosphorothionate (thionophosphate sulfone).—Freshly distilled diethoxythiophosphoryl chloride (17.3 g.) was added to a stirred mixture of 15 g. 2-hydroxydiethyl sulfone, 15 g. finely powdered anhydrous potassium carbonate, 0.2 g. copper powder and 5 ml. of anhydrous methyl ethyl ketone. The temperature of the mixture was maintained at 80° C. during the addition. The temperature was raised to 90° C. and the mixture was stirred for 4 hours. Methyl ethyl ketone was added, the mixture was filtered and the product was distilled in the falling-film molecular still, b.p. 145° C. (0.05 mm.), n_D^{29} 1.4667. The yield was 10 g. Elemental analysis: per cent calculated for $C_8H_{19}S_2O_5P$, P = 10.7; found, P = 10.6.

Paper Chromatography of Isomer Derivatives and Metabolites.—The two systems of reversed-phase paper chromatography which have been found useful in separating the various derivatives of the thiono and thiol isomers have been adequately described in previous papers (March *et al.* 1954, Metcalf *et al.* 1954). To review briefly, the first method (A) employs Whatman No. 1 paper uniformly impregnated with Dow Corning Corporation silicone 550 from a 5 per cent petroleum ether (*Skellysolve* B) solution. The developing solution is prepared by taking the upper layer of a mixture consisting of (by volume) 10 parts 95 per cent ethyl alcohol, 10 parts chloroform, and 6 parts water. The second method (B) employs Whatman No. 1 paper impregnated with propylene glycol from a 50 per cent ethyl alcohol solution and dried in air. The developing solvent is a mixture (by volume) of 8 parts *Skellysolve B* and 2 parts benzene, saturated with propylene glycol. The average R_f values and molar concentration of some of the derivatives of the isomers for 50 per cent inhibition of fly-brain cholinesterase are given in table 1.

Table 1.—The average R_f values on propylene glycol and silicone paper, and molar concentration for 50 per cent inhibition of fly-brain cholinesterase.

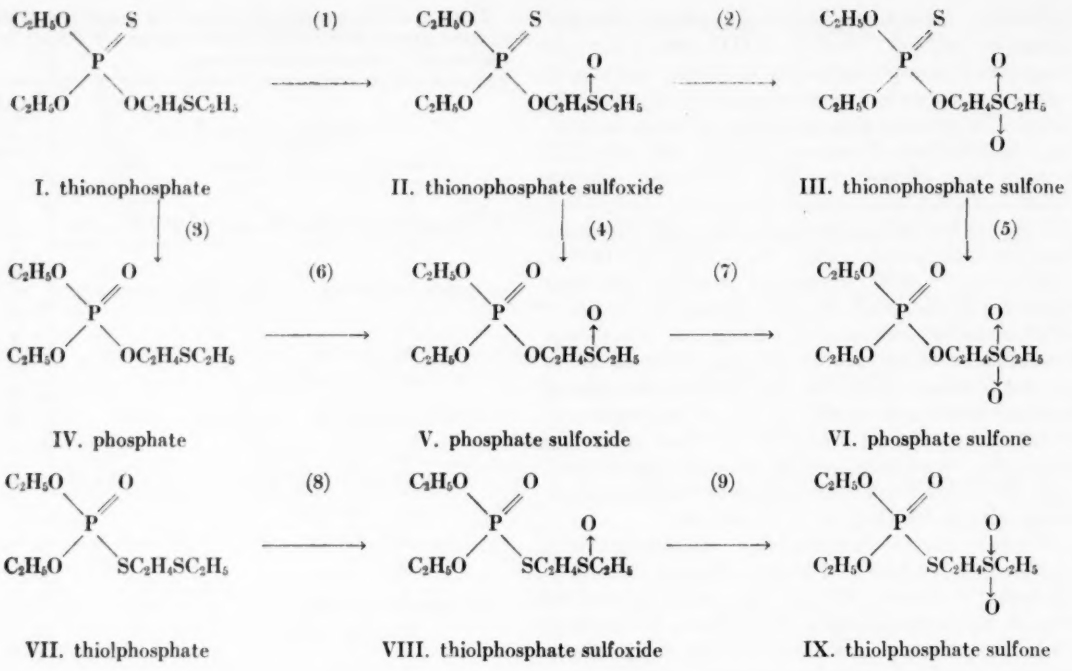
	Compound	R_f Glycol	R_f Silicone	Fly-Brain Cholinesterase Inhibition I_{50}
1.	$(C_2H_5O)_2P(S)OC_2H_4SC_2H_5$	0.99±0.01	0.05±0.01	2.2×10^{-4}
2.	$(C_2H_5O)_2P(O)SC_2H_4SC_2H_5$	0.80±0.02	0.69±0.02	3.5×10^{-6}
3.	$(C_2H_5O)_2P(O)OC_2H_4SC_2H_5$	0.75±0.06	0.75	2.4×10^{-8}
4.	$(C_2H_5O)_2P(S)OC_2H_4S(O)C_2H_5$	0.26±0.04	0.90	3.6×10^{-6}
5.	$(C_2H_5O)_2P(O)SC_2H_4S(O)C_2H_5$	0.04	0.95±0.02	1.5×10^{-6}
6.	$(C_2H_5O)_2P(O)OC_2H_4S(O)C_2H_5$	0	0.89±0.08	1.1×10^{-6}
7.	$(C_2H_5O)_2P(O)OC_2H_4S(O)C_2H_5$ (=O)	0	0.98	1.2×10^{-7}
8.	$(C_2H_5O)_2P(O)SC_2H_4S(O)C_2H_5$ (=O)	0	0.98	6.0×10^{-7}
9.	$(C_2H_5O)_2P(S)OC_2H_4S(O)C_2H_5$ (=O)	0	0.91	8.3×10^{-7}
10.	$(C_2H_5O)_2P(O)SH$	0 ±0.03	0.97	
11.	$(C_2H_5O)_2P(O)OH$	0		

The isolation of the metabolic products of the isomers from the cotton plant was carried out as follows. The bases of young cotton plants were treated with 10λ of either the P^{32}-labeled thiono or thiol isomer. After definite time intervals, the leaves were homogenized in an all-glass Potter-Elvejhem homogenizer, using 1 ml. of water for each 0.1 g. of leaf material. The aqueous portion of the homogenate was then separated by centrifugation, and the metabolic products were extracted into chloroform. The chloroform solution was concentrated under vacuum almost to dryness, and the residue was chromatographed by method B.

The method for scanning the paper chromatograms has been described previously (Fukuto & Metcalf 1954). Larger amounts of metabolic products were obtained by the same method, using sheets of paper instead of strips. The radioactive sections corresponding to each of the metabolites were cut out, diced, and extracted with acetone. The metabolite concentration in the acetone solution was determined by measuring the radioactivity of an aliquot in a planchet after removal of the solvent. The method for radioactive assay has been described previously (Metcalf *et al.* 1954).

The partition coefficients between water and chloroform of the isomers, their H_2O_2 oxidation products, and their hydrolysis products were determined with P^{32}-labeled products. The partition coefficients of the nonionic products were found to favor chloroform in the ratio of at least 20 to 1. The hydrolysis or ionic products obtained by the aqueous hydrolysis of diethoxy thiophosphoryl chloride, presumably diethyl phosphoric acid or diethyl thiophosphoric acid, partitioned completely in favor of water.

The technics for determination of the contact toxicity



of the oxidation products to the citrus red mite, *Metatetranychus citri* (McG.); the greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouché), and the common house fly, *Musca domestica* L., have been described elsewhere (Metcalf & Carlson 1950).

For the determination of the systemic action of the oxidative derivatives, the bases of young cotton plants (6-leaf stage) were treated with 10λ of material. After various time intervals leaves were picked, and Munger Cells were prepared with the greenhouse thrips, *Heliothrips haemorrhoidalis;* the two-spotted spider mite, *Tetranychus telarius* (L.); and the cotton aphid, *Aphis gossypii* Glover. Mortality was determined after 24 and 48 hours. A portion of the leaves was homogenized in an all-glass Potter-Elvejhem homogenizer with 10 times its weight in water, centrifuged, and the supernatant used to measure cholinesterase inhibition. The anticholinesterase activities were measured manometrically using 0.1 ml. of leaf homogenate, acetylcholine bromide 0.01 M, and housefly-head cholinesterase (Metcalf & March 1949).

The rates of hydrolysis were determined manometrically in the Warburg apparatus at 37° C. A bicarbonate-buffered solution (pH 7.4), 0.15 M in sodium chloride, 0.085 M in magnesium chloride, and 0.025 M in sodium bicarbonate, was used as the alkaline media. This buffered solution was made about 0.008 M in isomer derivative, and the rate of hydrolysis was followed by the evolution of carbon dioxide under constant agitation.

DISCUSSION.—The presence and relative ease of oxidation of the thiono sulfur and 2-ethylmercapto ether moieties in the thiono isomer and the 2-ethyl-mercapto ether moiety in the thiol isomer suggests that any metabolic changes of these isomers in biological systems might be oxidative in nature. The conversion of a thiono sulfur (P=S) to a phosphate oxygen (P=O) has been demonstrated by mild oxidation with nitric acid (Pishchimuka 1912). Dialkyl thioethers have been converted to the dialkyl sulfoxides and sulfones by a variety of oxidizing agents (Suter 1944). On the basis of an oxidative mechanism, seven possible nonionic products can be realized from both isomers as shown in the schematic diagram below. Oxidation of the thioether linkage in the thionophosphate (I) by steps 1 and 2 give, respectively, the thionophosphate sulfoxide (II) and the thionophosphate sulfone (III). The thionophosphate may also be oxidized by step 3 to give the phosphate (IV), which in turn may be converted by steps 6 and 7 to the phosphate sulfoxide (V) and the phosphate sulfone (VI). These two products may also be derived by steps 4 and 5 from the thionophosphate sulfoxide and sulfone. Oxidation of the thiolphosphate (VII) gives, in sequence, the thiolphosphate sulfoxide (VIII) by step 8, and the thiolphosphate sulfone (IX) by step 9. It is difficult to visualize the formation of any other oxidation products than the ones shown in the diagram. Hydrolysis of these oxidation products gives either *O,O*-diethyl phosphoric acid or *O,O*-diethyl thiophosphoric acid, both of which are nontoxic.

Mild hydrogen peroxide treatment of the P^{32}-labeled thionophosphate in acetone after 1 hour at 30° C. produced a compound which gave a spot on propylene glycol paper (method B) at R_f 0.32 (see Fig. 1). With further standing this compound gradually disappeared, and after 3 days the greater proportion of product was a compound which appears on propylene glycol paper at R_f 0.02. Besides the formation of this compound found at the origin, elemental sulfur precipitated out of the reaction mixture; this indicated the conversion of the thiono sulfur to the phosphate oxygen linkage. By a larger scale oxidation for 1 hour, using nonradioactive thionophosphate, a good yield of product was obtained which analyzed for the thionophosphate sulfoxide (II).

In view of the formation of sulfur after 3 days' oxidation, indicating that a phosphate was being formed, the phosphate sulfoxide (V) was prepared by mild oxidation with hydrogen peroxide for 1 hour at 30° C. from diethyl 2-ethylmercaptoethyl phosphate (IV). This compound V on propylene glycol paper gave an R_f of 0.02. In addition to the thionophosphate and phosphate sulfoxides, the phosphate sulfone (VI) was also prepared by the condensation of 2-hydroxydiethyl sulfone and diethoxyphosphoryl chloride in the presence of pyridine. This compound gave an R_f of 0 on propylene glycol paper. The sequence of reactions when the thionophosphate is oxidized by hydrogen peroxide appears to be steps 1, 4, and perhaps 7. The possibility of the formation of the thionophosphate sulfone (III) as a transitory intermediate cannot be ignored, however, since it, too, can release sulfur by oxidation. This compound was prepared by the condensation of 2-hydroxydiethyl sulfone and diethoxythiophosphoryl chloride and it showed an R_f of 0 on propylene glycol paper.

Chromatographic analysis of the metabolic products of the thionophosphate from the cotton plant gave curves very similar to that obtained by hydrogen peroxide oxidation (see Fig. 2). The chromatogram of the metabolite after 1 day in the plant showed the predominance of a compound at R_f 0.29. This spot corresponds, within experimental error, to that of the thionophosphate sulfoxide. This peak gradually diminished until after 8 days the major metabolite was one found at R_f 0.02, which corresponds to the phosphate sulfoxide and the thionophosphate sulfone. The small peaks found at R_f 0.8 or greater are probably due to unmetabolized thionophosphate, or possibly to the phosphate (IV).

Mild hydrogen peroxide oxidation of P^{32}-labeled thiolphosphate appeared to give a single product. The chromatograms in figure 1 show that only one peak at R_f 0.03 is obtained after 3 hours', 22 hours', and 3 days' oxidation at 30° C. A good yield of a compound which analyzed correctly for the thiolphosphate sulfoxide (VIII) was obtained when the thiolphosphate was oxidized with hydrogen peroxide for 3 hours at 30° C. This compound gave an R_f of 0.02 on propylene glycol paper. In addition, the corresponding thiolphosphate sulfone (IX) was prepared by the permanganate oxidation of the thiolphosphate. This compound gave an R_f of 0 on propylene glycol paper. It is possible that the single peaks in figure 1, especially those obtained after 22 hours' and 3 days' oxidation, actually represent two compounds in view of the similar R_f values of the thiolphosphate sulfoxide and thiolphosphate sulfone. Chromatographic analysis of the metabolic products of the thiolphosphate from the cotton plant gave curves very similar to that obtained by hydrogen peroxide oxidation (see Fig. 3). Only one peak was found at R_f 0.02, which corresponds to the thiolphosphate sulfoxide and sulfone. The peaks found at R_f 0.8–0.9 are probably due to unmetabolized thiolphosphate.

A comparison was made of fly-brain cholinesterase inhibition by the P^{32}-labeled plant metabolites and by the corresponding P^{32}-labeled hydrogen peroxide oxidation products and synthetic products. The molar concentrations for 50 per cent inhibition are shown in table 2. The

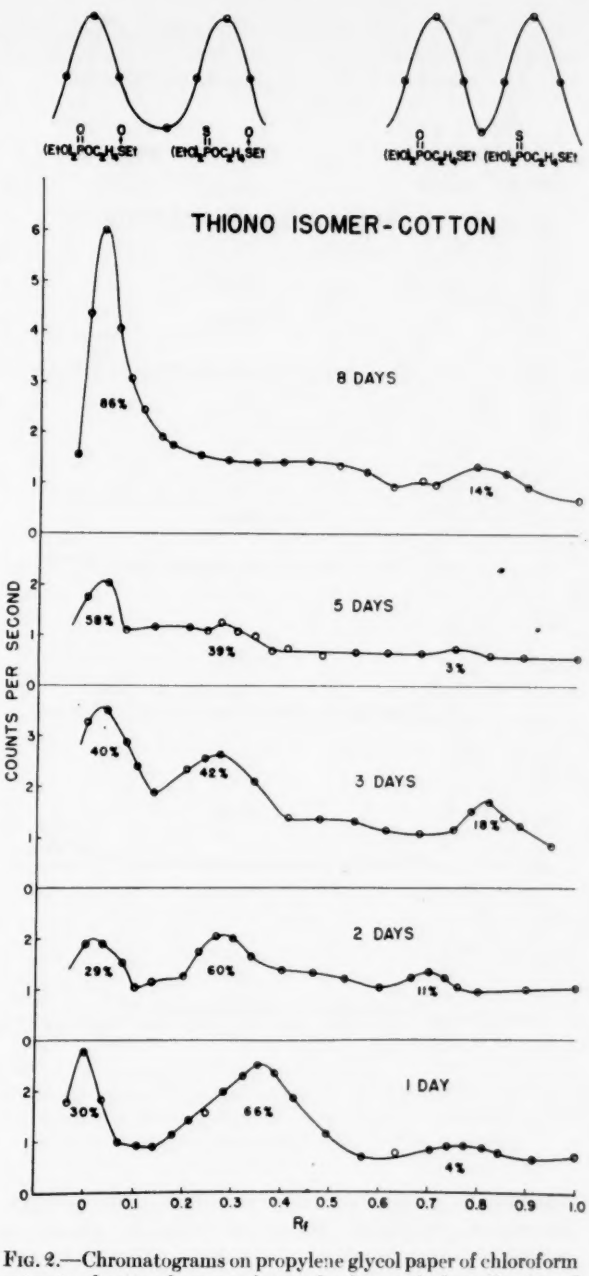


FIG. 2.—Chromatograms on propylene glycol paper of chloroform extracts of cotton leaves at intervals after topical application of P^{32}-tagged thiono isomer at base of stem.

values for 50 per cent inhibition between each metabolite and its chromatographically identical oxidation product agree fairly well. The values for the synthetic products, shown by numbers 3, 4, 7, and 11, appear to be somewhat lower. The purification of these compounds proved difficult because of their tendency to decompose with heat, and the differences found are probably due to small amounts of impurities produced in distillation.

Since the systemic activity of the thionophosphate and thiolphosphate is due to metabolic products, the synthetic compounds selected as the probable metabolites

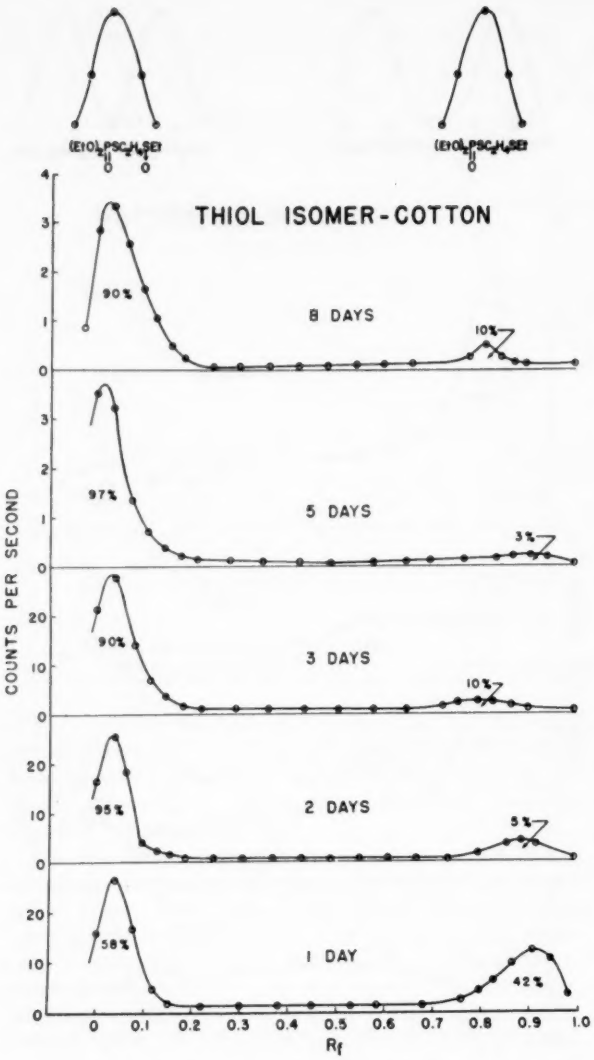


FIG. 3.—Chromatograms on propylene glycol paper of chloroform extracts of cotton leaves at intervals after topical application of P^{32}-tagged thiol isomer at base of stem.

should also possess systemic properties. The systemic actions of phosphate, phosphate sulfoxide, phosphate sulfone, thionophosphate sulfoxide, thionophosphate sulfone, thiolphosphate sulfoxide, and thiolphosphate sulfone were determined by removing leaves from cotton plants treated at the base, and infesting these with mites, thrips, and aphids in Munger Cells. The data are presented in table 3. The thionophosphate sulfoxide, thiolphosphate sulfoxide, and thiolphosphate sulfone showed extremely good systemic activity. The thiolphosphate sulfoxide and sulfone were highly active even at 4 weeks after application to the stem of the cotton plant. The phosphate and phosphate sulfone showed no activity. The small degrees of activity of the phosphate sulfoxide and thionophosphate sulfone were disappointing since the chromatographic evidence and cholinesterase inhibition indicated that they might be the final metabolites of the thiono isomer. There is still some question concerning the true nature of this final metabolite, which will be discussed later.

Table 2.—Molar concentration of oxidation and metabolic products of thiono and thiol isomers for 50 per cent inhibition of fly-brain cholinesterase.[a]

PRODUCT	I_{50}	R_f
1. Thiolphosphate metabolite	6×10^{-6}	0.03
2. 3-Hour H_2O_2 oxidation thiolphosphate	6×10^{-6}	0.03
3. Thiolphosphate sulfoxide	1.5×10^{-6}	0.04
4. Thiolphosphate sulfone	6.0×10^{-7}	0
5. Thionophosphate metabolite	5×10^{-6}	0.02
6. 3-Days H_2O_2 oxidation thionophosphate	2×10^{-6}	0.02
7. Phosphate sulfoxide	1.1×10^{-6}	0
8. Thionophosphate sulfone	8.3×10^{-7}	0
9. Thionophosphate metabolite	8×10^{-6}	0.29
10. 1-Hour H_2O_2 oxidation thionophosphate	1.1×10^{-5}	0.32
11. Thionophosphate sulfoxide	3.6×10^{-6}	0.26

[a] The R_f values on propylene glycol paper are listed in the second column for comparison.

Evaluation of the toxicity of the oxidation products of the isomers to insects, mites, and the white mouse showed that the phosphate sulfoxide was in general the least toxic (Table 4). The lack of toxicity of the phosphate sulfoxide and sulfone is difficult to understand in view of their rather high *in vitro* anticholinesterase activity (see Table 1).

The rates of hydrolysis of the oxidation products were determined manometrically in dilute sodium bicarbonate solution at the initial pH of 7.9 .The mole per cent hydrolyzed after 60 minutes at 37° C. is given as follows: thiolphosphate sulfoxide, 1.6; thionophosphate sulfoxide, 6.2; thionophosphate 9.4; thiolphosphate sulfone, 9.7; thionophosphate sulfone, 10.1; phosphate sulfoxide, 13.7; thiolphosphate, 19.6; and phosphate, 39.0. The reaction rates showed no definite order kinetics and the curves all leveled off after about 40 minutes. The percentage hydrolyzed after 60 minutes is given for comparison. Stability to hydrolysis in aqueous solution is essential for long-life systemic activity since the hydrolyzed products are nontoxic. The rapid rate of hydrolysis of the phosphate explains its poor insecticidal and systemic activity, in spite of its high *in vitro* anticholinesterase activity. The thiolphosphate sulfoxide, thiolphosphate sulfone and thionophosphate sulfoxide are relatively stable to hydrolysis, and these compounds demonstrated long-life systemic activity.

On the basis of the evidence obtained from paper chromatography, cholinesterase activity, systemic activity, mammalian and insect toxicities, it appears that the thionophosphate is first converted to the thionophosphate sulfoxide and then to either the phosphate sulfoxide or thionophosphate sulfone. Both phosphate sulfoxide and thionophosphate sulfone showed poor systemic activity, but the relatively greater toxicity of the thionophosphate sulfone to insects and mites might possibly favor the sulfone as the final major metabolite. On the same basis the thiolphosphate is first converted to the sulfoxide by plants. The evidence points to the sulfoxide as the sole metabolite, but the possibility that the sulfone is also present must be considered, since both of these compounds have the same R_f values.

In a few isolated cases, radioautographs of propylene glycol chromatograms of metabolic products of the thiono and thiol isomers in plants showed an additional radioactive spot slightly above the spot of application,

Table 3.—Systemic activity of derivatives of systox isomers in the cotton plant.[a]

Compound	Time After Treatment	Cholinesterase % Inhibition Leaf Homogenate		Per Cent Mortality in Munger Cells					
				Thrips		Mites		Aphids	
		10 mg.	1 mg.	24 Hours	48 Hours	24 Hours	48 Hours	24 Hours	48 Hours
Phosphate sulfoxide	2 hours	0							
	4 hours	15		0	0	20	55	0	0
	24 hours	100	12	15		35		0	
	48 hours	20	0	0	0	30	30	0	90
	1 week	4	0	0	0	0	15	0	100
Thionophosphate sulfoxide	2 hours	0							
	4 hours	0							
	24 hours	0		65		95		100	
	48 hours	90	0	100		70	95	100	
	1 week			100		100		100	
	15 days	37	15	60		0		100	
	21 days	24		0	65	55	100	100	
	28 days	0		45	100	20	80	100	
Thiolphosphate sulfoxide	2 hours	0							
	4 hours	40		100		80	100	100	
	24 hours	99	73	100		100		100	
	1 week	94	87	100		85	100	100	
	2 weeks	94	28						
	15 days	99	86	100		50		100	
	3 weeks	95	46	95	100	30	100	100	
	4 weeks	88	25	80	100	85	100	100	
Phosphate	2 hours	22		0	0	10	10	0	0
	4 hours	97		5	5	15	15	0	0
	24 hours	22		0	0	0	0	0	90
Phosphate sulfone	2 hours	0		0	0	5	5		
	4 hours	0		0	0	0	5	0	0
	24 hours	0		0	0	0	35	0	100
	7 days			0	0	0	0		
Thiolphosphate sulfone	2 hours	5							
	4 hours	18	15	0	0	15	25	90	100
	24 hours	47	20	55	100	80	100	100	
	48 hours	92	55	100		100		100	
	1 week			100		90	100	100	
	2 weeks	79	29	80	100	50	100	100	
	3 weeks	100	60	100		100		100	
	4 weeks	72	16						
Thionophosphate sulfone	2 hours	0							
	4 hours	0							
	24 hours	2							
	48 hours	0							
	1 week	0							
	3 weeks	0							

[a] Five microliters applied at base of plant stems.

possibly because of differences in amounts of propylene glycol on the paper, as shown in figure 4. The chromatogram on the right, showing metabolites obtained from the lemon plant 4 days after treatment with thiono isomer, shows a very small spot slightly above the one at the origin which constituted the greatest proportion of the metabolic products. This small spot actually represents less than 1 per cent of the total metabolites as shown by radioassay. These two spots at the origin probably represent the thionophosphate sulfone and phosphate sulfoxide. The third spot near the middle has been shown to be the thionophosphate sulfoxide. The chromatogram on the left shows the metabolic products obtained from the lemon plant 4 days after treatment with the thiol isomer. Here, too, a second metabolite, representing only a few per cent of the total metabolic products as shown by radioassay, appeared slightly above the origin. These two spots probably represent the thiolphosphate sulfoxide and sulfone. The smear at the top could not be detected by the Geiger tubes and probably represents traces of unchanged thiol isomer.

Work is in progress in obtaining infrared spectra of the metabolic products obtained from plants. The infrared spectra of the seven possible oxidation products have been recorded and details of the spectra will be published at a later date along with those of the metabolic products.

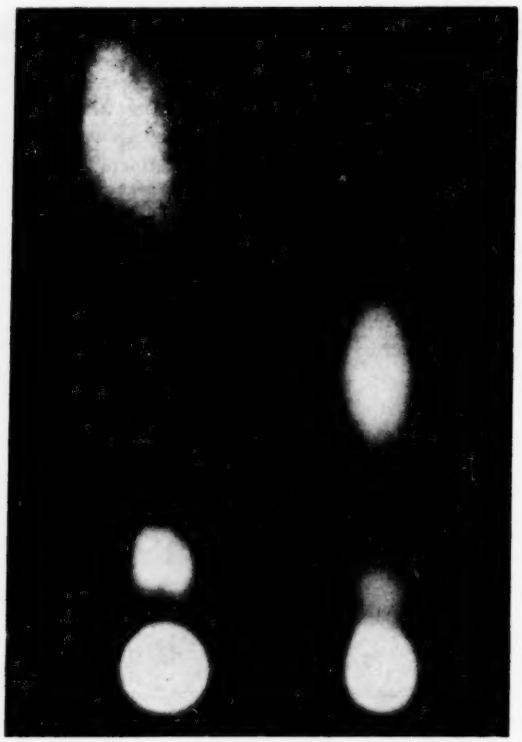


FIG. 4.—Radioautographs of lower half of propylene glycol chromatograms, showing metabolites of the thiono isomers (right) and thiol isomers (left) after 4 days in the lemon plant.

A comparison of these spectra should give the final answer to the metabolic fate of the Systox isomers in plants and animals.

SUMMARY AND CONCLUSIONS.—The seven oxidation products of the thiono and thiol isomers, *O,O*-diethyl *O*-ethyl-2-sulfinylethyl phosphorothionate (thionophosphate sulfoxide), *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate (thiolphosphate sulfoxide), *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphate (phosphate), *O,O*-diethyl *O*-ethyl-2-sulfinylethyl phosphate (phosphate sulfoxide), *O,O*-diethyl *O*-ethyl-2-sulfonylethyl phosphate (phosphate sulfone), *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate (thiolphosphate sulfone), and *O,O*-diethyl *O*-ethyl-2-sulfonylethyl phosphorothionate (thionophosphate sulfone) have been synthesized and some of their properties were compared with the metabolic products of the Systox isomers obtained after topical applications to the base of the cotton plant. The comparison of the results obtained from paper chromatography, cholinesterase activity, systemic activity, mammalian and insect toxicities of the oxidation and metabolic products indicates that the thiono isomer of Systox is converted to the thionophosphate sulfoxide, which is then converted to the thionophosphate sulfone or phosphate sulfoxide, or to both. A similar comparison of the oxidation and metabolic products of the thiol isomer indicates that it is converted to the thiolphosphate sulfoxide and then possibly to the thiolphosphate sulfone.

Table 4.—Toxicity of isomer derivatives to insects, mites and the white mouse.

COMPOUND	WHITE MOUSE INTRAPERITONEAL (MG./KG.)	HOUSE FLY TOPICAL 24 HOURS $LD_{50}\gamma$/FLY	LC_{50} IN PER CENT CONCENTRATION	
			Adult Mites[a]	Thrips[b]
Phosphate sulfoxide	350	8.7	>0.1	0.1
Phosphate sulfone	27	3.7	0.04	0.3
Thionophosphate sulfoxide	75	2.0	0.04	0.028
Thionophosphate sulfone	75	1.2	0.05	0.0020
Phosphate	20	0.74	<0.1	0.038
Thiolphosphate sulfoxide	12	0.75	0.0013	0.0023
Thiolphosphate sulfone	10–15	1.2	0.0014	0.0028

[a] Citrus red mites.
[b] Greenhouse thrips.

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Metabolism of Systox in the White Mouse and American Cockroach[1,2]

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The systemic insecticide Systox[3] is a mixture of two isomers the thiono isomer, *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate (thionophosphate), and the thiol isomer, *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate (thiolphosphate) (Schrader 1952, Gardner & Heath 1953, Henglein *et al.* 1954, Fukuto & Metcalf 1954). These isomers have been shown to produce the typical symptoms of anticholinesterase agents in mammals and insects and to be potent inhibitors of cholinesterase *in vivo* and *in vitro* (Schrader 1952, Du Bois & Coon 1952, Wirth 1953, Heath *et al.* 1953, Metcalf *et al.* 1955a). Almost no information has been published, however, on the metabolism and fate of these compounds in animals. Such information is necessary to complete the knowledge of their toxicology and mode of action, and in addition must be considered in order to interpret the toxicology of their residues in plants intelligently. The following studies were therefore undertaken concurrently with others on the mode of action and metabolism and fate of these compounds in plants, and on their chemistry and toxicology (Metcalf *et al.* 1954b and 1955, and Fukuto *et al.* 1955), in order to evaluate these factors in a mammal, the white mouse, and in an insect, the American cockroach, *Periplaneta americana* (L.).

MATERIALS AND METHODS.—The P^{32}-labeled preparations of the thiono and thiol isomers and the compounds prepared as possible metabolites which were used in these studies were synthesized as described by Fukuto *et al.* (1955). Solutions in propylene glycol were employed for *in vivo* experiments, and in ethyl alcohol or dioxane for *in vitro* experiments. All solutions were stored under refrigeration to minimize spontaneous changes.

The techniques used in radioassay, paper chromatography, partitioning between immiscible solvents, house fly cholinesterase inhibition, and radioautography have been previously described (Metcalf & March 1952, Metcalf *et al.* 1954a and 1954b, March *et al.* 1954, Fukuto & Metcalf 1954, Fukuto *et al.* 1955). The radioautographs were examined, and density scans were prepared by means of a photoelectric densitometer.[4]

In vivo experiments with mice were performed on single animals. Oral administration of the propylene-glycol solutions of the isomers was accomplished by means of a micrometer-driven syringe (March & Metcalf 1949) and a ball-tipped needle. The treated mice were kept singly in metabolism cages modified after the method of Hansard & Comar (1953) for the separate collection of urine and feces. On sacrificing, the mice were anesthetized with chloroform and the liver, kidneys, and stomach together with approximately 2 inches of intestine were removed *in toto*. For blood assay as much blood as possible was withdrawn into a citrated syringe by heart puncture. The tissues were homogenized in water in an all-glass Potter-Elvejhem homogenizer and together with the urine were extracted three times with equal volumes of chloroform. Following separation by centrifugation, the chloroform or water extracts were evaporated for radioassay or concentrated for preparation of paper chromatograms.

For *in vitro* experiments with mouse tissues, a mouse was lightly anesthetized with chloroform, and the liver, kidneys, thigh muscle, and brain were removed, washed in cold phosphate buffer, and sectioned as thinly as possible. Approximately equal amounts of the cut slices were transferred directly into Warburg flasks containing 2.3 ml. of M/15 Na_2HPO_4 buffer (pH 8.6), 0.3 ml. of 0.1M sodium citrate in phosphate buffer containing 6.23 mg. of ATP per ml., 0.3 ml. of 0.1M sodium fumarate in phosphate buffer, 0.1 ml. of 20 per cent (weight to volume) KOH in the center well and 0.1 ml. of 1×10^{-2} M thiono or thiol isomer in ethyl alcohol or dioxane. The flasks were oxygenated for 5 minutes; they were then closed and metabolism was permitted to proceed for 2 to 4 hours. The contents of the flasks were homogenized and extracted 3 times with 3-ml. aliquots of chloroform, and the concentrated extracts were used for preparation of chromatograms.

The *in vivo* and *in vitro* experiments with the American cockroach were similar to those for the white mouse except in the following respects. The isomers in propylene glycol were applied topically on the ventral cervical membrane, or were injected orally or through the mesothoracic spiracle by means of the micrometer-driven applicator noted above. Pooled tissues from 10 male roaches were used in all *in vivo* studies. In the *in vitro* experiments the whole guts, nerve cords, or thoracic muscles from 5 male roaches were used instead of tissue slices as in the mouse.

The *in vitro* anticholinesterase activities of the chromatographically separated compounds were determined as follows. After radiometric location and quantitative estimation of the separated compounds (Fukuto & Metcalf 1954), the areas containing radioactive compounds were cut from the chromatograms and diced directly into Warburg flasks containing the necessary ingredients for the determination of inhibition of house fly head cholinesterase (Metcalf *et al.* 1954b). Inhibitory activity was established by comparison with blank runs containing diced areas of the chromatograms showing no radioactivity.

EXPERIMENTAL RESULTS—*White Mouse.*—The distribution of the thiono and thiol isomers and their metabolites in the stomach and duodenum, liver, kidney, blood, and urine of the white mouse, and the partitioning of the radioactive compounds extracted from these tissues between chloroform and water at various time intervals after their oral administration in propylene-

PAID PAPER.
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[2] Part III of a series of papers on the mode of action of Systox. This work was supported in part by grants from the U. S. Atomic Energy Commission, Contract AT (11-1) 34, Project 6, and from the Chemagro Corporation, New York, N. Y.
[3] Trade-mark, Chemagro Corporation, New York, N. Y.
[4] Photovolt Corporation New York, N. Y.

Table 1.—Distribution of thiono and thiol isomers and metabolites in mouse tissues and their partition between chloroform and water at various intervals after oral administration in propylene-glycol solution.

Time Sacrificed Hours	Dose			Partition Fraction	Per Cent Total Dose/Tissue/Mouse				
	Sex	γ/g.	Total γ		Stomach and Duodenum	Liver	Kidney	Blood[a]	Urine
				Thiono Isomer and Metabolites					
½	♂	25	825	$CHCl_3$	4.8	1.3	0.56	—	—
				H_2O	0.55	2.8	0.99	—	—
½	♀	50	1000	$CHCl_3$	20	0.37	0.08	—	0.41
				H_2O	0.97	1.5	0.33	—	2.7
1	♂	25	750	$CHCl_3$	—	0.26	0.06	0.07	0.47
				H_2O	—	3.3	0.81	0.52	7.3
2	♀	50	1000	$CHCl_3$	6.1	0.40	0.09	—	2.5
				H_2O	0.60	1.6	0.34	—	16
4	♀	25	600	$CHCl_3$	0.31	0.07	Trace	—	5.8
				H_2O	0.58	0.79	0.18	—	34
24	♀	26	550	$CHCl_3$	0.08	0	Trace	—	6.4
				H_2O	0.32	0.39	0.06	—	58
24	♂	12.5	375	$CHCl_3$	—	—	—	—	3.2
				H_2O	—	—	—	—	71
				Thiol Isomer and Metabolites					
½	♀	50	1000	$CHCl_3$	4.6	0.29	0.08	—	—
				H_2O	0.75	1.2	0.58	—	—
1[b]	♀	50	1000	$CHCl_3$	1.6	0.43	0.05	—	—
				H_2O	0.46	0.12	0.47	—	—
2[c]	♀	25	750	$CHCl_3$	—	0.09	0.01	0	—
				H_2O	—	2.6	0.59	0.28	—
4[c]	♀	24	500	$CHCl_3$	0.37	0.74	0.08	—	1.5
				H_2O	0.44	1.3	0.29	—	16
24	♀	24	500	$CHCl_3$	0	0.11	0.24	—	4.0
				H_2O	0.37	0.45	0.11	—	49
24	♀	12.5	375	$CHCl_3$	—	—	—	—	5.4
				H_2O	—	—	—	—	69

[a] Portion of total blood volume only, obtained by heart puncture.
[b] Died ½ hour.
[c] Prostrate.

glycol solution, are shown in table 1. Since the thiono and thiol isomers and their toxic metabolic products partition almost completely in favor of chloroform, and the ionic, nontoxic degradation products partition in favor of water (Fukuto *et al.* 1955), these results indicate considerable information concerning the metabolic degradation of the isomers as well as concerning their absorption and distribution.

The administered dose is rapidly absorbed, metabolized, and eliminated. Only 6 per cent or less of the administered activity is found in the stomach and duodenum after 1 to 2 hours, and the administered dose has almost entirely disappeared from these tissues after 4 hours. Small amounts may pass through the intestines and be eliminated in the feces. This is not the major route of elimination, however. Radioassay of a portion of the 24-hour feces yielded less than 1 per cent of the administered dose, some of which may have been derived from contamination of the feces with urine. The large amounts of chloroform-partitioning compounds in the stomach and duodenum indicate that degradation to water-partitioning compounds is of little consequence here. The relatively constant amount of residual water-partitioning compounds suggests that these ionic products are not so rapidly absorbed as the nonionic compounds, and that they probably account for the small amount of activity found in the feces.

Within the relatively short interval of the first 2 hours, considerably more than half of the recovered activity in the liver, kidney, blood, and urine is normally found in the water fraction and by 24 hours even larger amounts of water-partitioning compounds are present, in some cases to the complete exclusion of chloroform-partitioning compounds. This rapid formation of water-partitioning ionic degradation products suggests that they are produced by biochemical processes which are distinct from

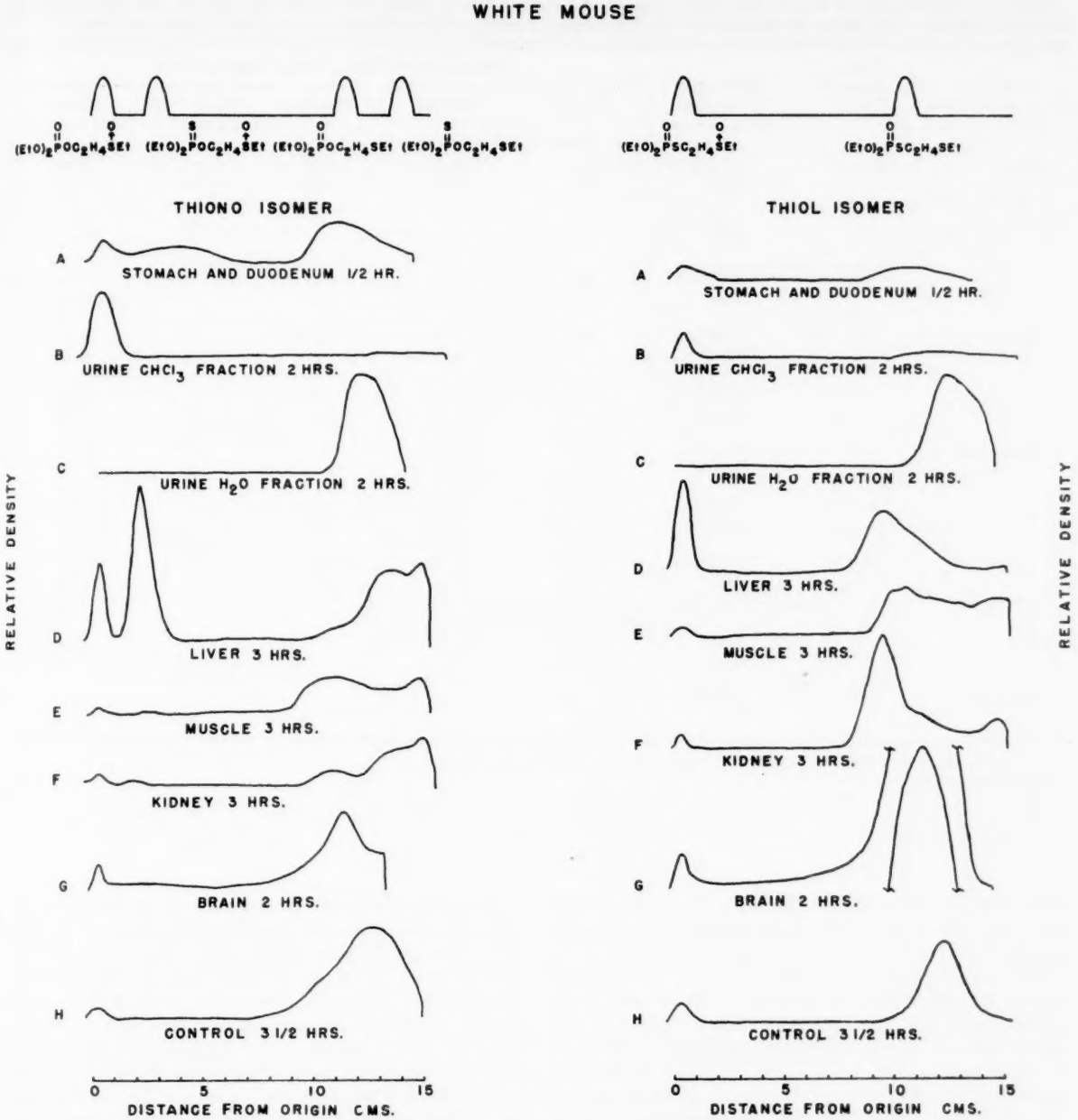

FIG. 1.—*In vivo* (A-C curves) and *in vitro* (D-H curves) metabolism of thiono and thiol isomers in various tissues of the white mouse, based on density scans of radioautographs prepared from chromatograms of chloroform-partitioned (A, B, D-H curves) and water partitioned (C curves) extracts of the tissues. (A, B, D-H curves, propylene-glycol paper; C curves, silicone paper.)

those producing the biologically active metabolities, and that they are not simply the end products of a chain of biochemical processes but may be produced by degradation of the thiono or thiol isomers themselves or their toxic metabolites.

The liver, as one would expect, contains large amounts of radioactive compounds, because the absorbed compounds after oral administration must pass through it by way of the hepatic portal system. In the case of the thiono isomer, the ratio of chloroform to water-partitioning compounds is relatively constant in the blood, kidney, and urine after the absorbed compounds have passed through the liver. This suggests that the liver is the major site of degradation to water-partitioning compounds. The data for the thiol isomer indicate that degradation continues following passage through the liver, since this ratio continues to decrease from liver to urine. It is most logical to assume that the liver is the most active tissue in this respect, but that degradation of both isomers and their toxic metabolites is a continuous process

Table 2.—Distribution of thiono and thiol isomers and metabolites in American cockroach tissues and their partition between chloroform and water at various intervals after administration of 25γ per roach in propylene-glycol solution.

Mode of Administration	Time Sacrificed Hours	Partition Fraction	Per Cent of Total Dose/Tissue/Roach				
			Foregut	Midgut	Hindgut	Whole Gut	Blood[a]
			Thiono Isomer and Metabolites				
Oral	1–2	$CHCl_3$	45	2.2	3.5	—	0.064
		H_2O	12	1.9	0.76	—	0.31
	4	$CHCl_3$	—	—	—	98	—
		H_2O	—	—	—	9	—
	5–6	$CHCl_3$	26	5.5	1.4	—	1.2
		H_2O	4.4	2.0	4.1	—	0.88
Topical	1–2	$CHCl_3$	40	1.0	0.44	—	1.6
		H_2O	1.8	0.40	0.56	—	0.18
	5–6	$CHCl_3$	26	1.7	1.0	—	0.80
		H_2O	3.4	0.92	2.5	—	0.48
Spiracular	4[b]	$CHCl_3$	—	—	—	16	—
		H_2O	—	—	—	5	—
	16–17[c]	$CHCl_3$	0.44	0	0.011	—	0.040
		H_2O	0.64	0.64	1.3	—	0.18
	16–17[d]	$CHCl_3$	1.0	0.60	3.8	—	0.26
		H_2O	1.9	1.1	32	—	0.48
			Thiol Isomer and Metabolites				
Oral	4	$CHCl_3$	—	—	—	83	—
		H_2O	—	—	—	16	—
Spiracular	4[e]	$CHCl_3$	—	—	—	12	—
		H_2O	—	—	—	10	—

[a] Portion of total blood volume only, obtained by absorption on filter paper from haemocoele.
[b] Mortality 50 per cent.
[c] Alive.
[d] Dead.
[e] Mortality 100 per cent.

until they are eliminated in the urine. It will be shown later that the liver is the most active of the tissues examined *in vitro* in producing the toxic metabolites.

The major pathway of elimination is through the urinary system. Within 24 hours, 50 to 70 per cent of the administered dose is eliminated in the urine and 90 per cent or more of the eliminated compounds are in the form of water-partitioning ionic degradation products. The rapidity of elimination depends in part on the amount of the administered dose and upon the toxic effects on the animal, larger and more toxic doses being eliminated less rapidly. Within the limitations of variation in the data in table 1, both isomers appear to be metabolized and eliminated at about the same general rate.

The rapid elimination of the thiono and thiol isomers and their metabolites from the mouse made it difficult to follow the progression of biochemical changes in the various tissues *in vivo* even by paper chromatography and radioassay of the tissue extracts. Thus the metabolism of these compounds was examined *in vitro* as well, with more easily apparent results being obtained. Typical results of *in vivo* and *in vitro* experiments in the form of density scans of radioautographs prepared from paper chromatograms of the chloroform and water extracts of various tissues are shown in figure 1.

The same metabolites are produced in the mouse *in vitro* as *in vivo* (compare A and D curves, figure 1). The chloroform-partitioning compounds extracted from the urine produce, in the case of each isomer, a single chromatographically separated metabolite peak and a much smaller peak for the unchanged isomer (see B curves, figure 1). The C curves (figure 1) show a single peak as well for the water-partitioning ionic metabolites from both isomers. Thus, both the thiono and the thiol isomers are almost entirely metabolized to other compounds before excretion. Ten per cent or less of the excreted compounds are chloroform-partitioning metabolites, and 90 per cent or more are water-partitioning ionic metabolites (see table 1). Only trace amounts of each are excreted unchanged.

The D, E, F, G, and H curves (figure 1) indicate the relative *in vitro* activities of liver, muscle, kidney, and brain in producing active metabolites. The liver is obviously the most active tissue in this respect. Muscle, kidney, and brain show little or no activity based on a comparison of the curves for these tissues (E, F, and G curves, figure 1) with the control curves (H curves, figure 1). The presence of a small but significant peak 2 to 3 cm. from the origin in the muscle and kidney curves for the thiono isomer shows a definite but limited converting

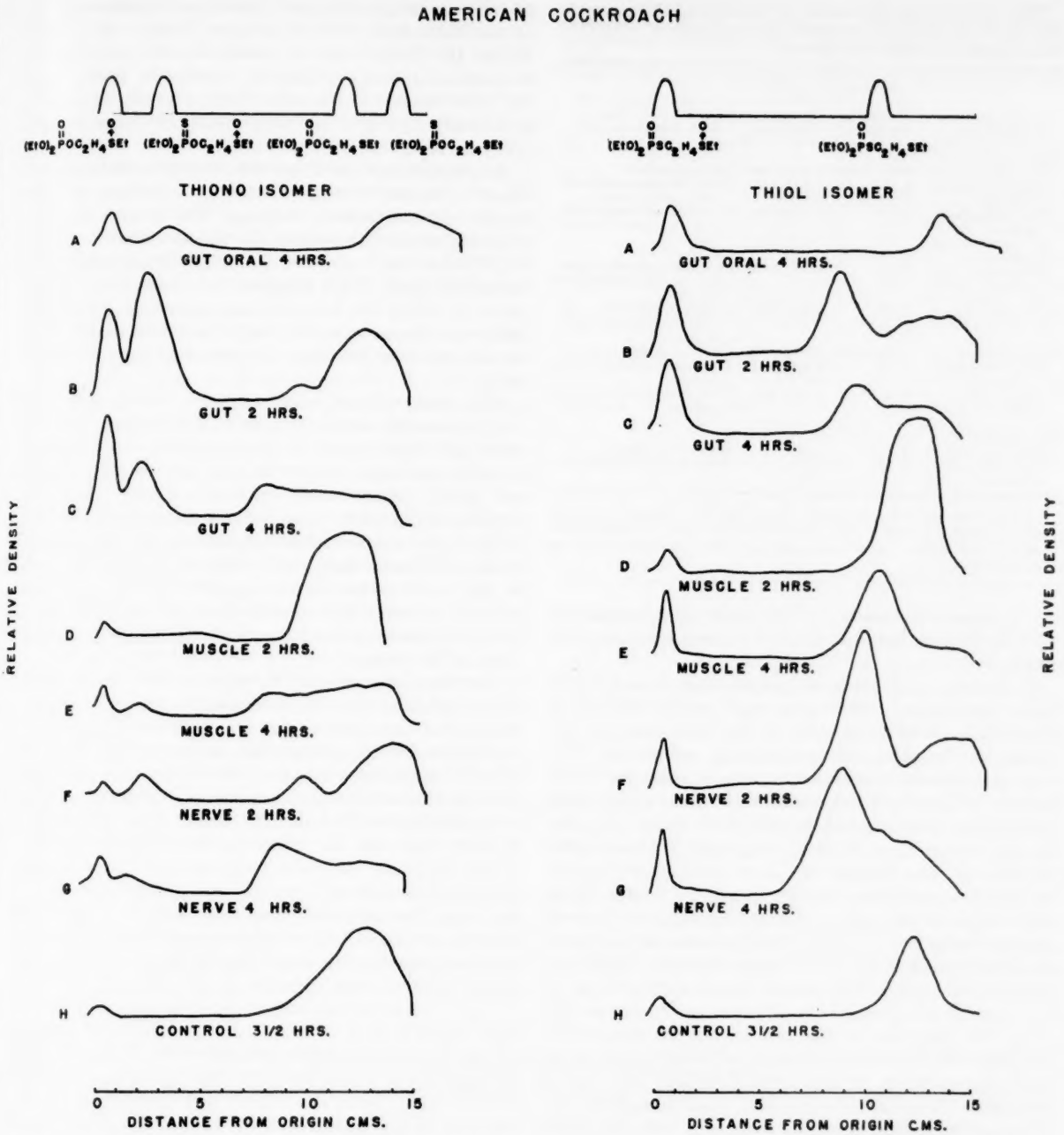

FIG. 2.—*In vivo* (A curves) and *in vitro* (B-H curves) metabolism of thiono and thiol isomers in various tissues of the American cockroach, based on density scans of radioautographs prepared from chromatograms of chloroform- and water-partitioned extracts of the tissues. (A-H curves, propylene-glycol paper; chloroform fraction.)

activity in these two tissues. This peak is not apparent for brain tissue, however. The presence of only a single peak for the thiol isomer makes it impossible to verify a similar limited activity for the tissues with this isomer, but one may assume that comparable activities occur. There is no indication of activity for brain in either case.

American Cockroach.—Because of time limitations and loss of activity in the P^{32}-preparation by normal decay, comparable data on the distribution and partitioning between chloroform and water for the thiono and thiol isomers and their metabolites in various tissues of the American cockroach were not obtained as for the white mouse. Fragmentary data on the distribution and partitioning of these compounds after various time intervals following oral, topical, and spiracular administration in propylene-glycol solution are given for the gut and blood in table 2. Although by no means complete, the data indicate that the rate of metabolism and elimination is

Table 3.—Average R_f values for thiono and thiol isomers and their metabolites prepared synthetically or extracted from mouse and cockroach tissues.[a]

Synthetic Compound	Average R_f Value: Synthetic Compound	Average R_f Value: Extracted Compound	Possible Identity of Compound Extracted from Animal Tissues
$CHCl_3$-Partitioning			
Thionophosphate	0.99±0.01	0.99±0.06	Thionophosphate
Thionophosphate sulfoxide	0.26±0.04	0.24±0.08	Thionophosphate sulfoxide
Thionophosphate sulfone	0.0	0.04±0.02	Phosphate sulfoxide or sulfone or thionophosphate sulfone
Phosphate	0.75±0.06	.72	Phosphate
Phosphate sulfoxide	0.00	0.04±0.02	Phosphate sulfoxide or sulfone or thionophosphate sulfone
Phosphate sulfone	0.00	0.04±0.02	Phosphate sulfoxide or sulfone or thionophosphate sulfone
Thiolphosphate	0.80±0.02	0.86±0.10	Thiolphosphate
Thiolphosphate sulfoxide	0.04	0.06±0.03	Thiolphosphate sulfoxide or sulfone
Thiolphosphate sulfone	0.0	0.06±0.03	Thiolphosphate sulfoxide or sulfone
H_2O-Partitioning			
Diethyl phosphate	1.0	1.0	Diethyl phosphate
Diethyl thiophosphate	0.97	1.0	Diethyl phosphorothioate

[a] Chloroform-partitioning compounds chromatographed on propylene-glycol paper with toluene-petroleum ether as mobile solvent; water-partitioning compounds chromatographed on silicone paper with chloroform-ethanol-water as mobile solvent.

not so rapid in the roach as in the mouse for a comparable dose by weight, but that in gross aspects they are very similar.

Both isomers and their metabolites are degraded into water-partitioning ionic compounds which increase in quantity with time, together with a corresponding decrease in the chloroform-partitioning compounds. The rate and amount of metabolism depend upon the living insect, because both chloroform- and water-partitioning metabolites accumulated in the dead insect to a far greater extent than in the living insect 16 hours after application. The foregut exhibits a marked selective absorption of the thiono isomer, quantities isolated from that tissue being approximately the same for topical application to the cervical membrane as for oral administration. Similar results were obtained with TEPP by Roan *et al.* (1950). The thiono isomer and some or all of its metabolites are able to diffuse from the gut as well, since the quantities of that isomer and its metabolites present in the blood are approximately equal after either topical application or oral administration. The thiono isomer and its metabolites gradually pass through the gut, the quantities found increasing posteriorly with time while decreasing anteriorly. The formation of water-partitioning metabolites increases with passage through the gut for larger amounts of these ionic metabolites are found in the hindgut than midgut and in the midgut than in the foregut 5 to 6 hours after administration. The fact that the blood contains a far greater proportion of chloroform- to water-partitioning compounds at comparable times then does the gut, suggests that the gut is the principal site of degradation and pathway for elimination. The data in table 2 indicate that considerations similar to those above may also be involved for the thiol isomer.

The *in vivo* and *in vitro* metabolism of the chloroform-partitioning compounds from cockroach gut, muscle, and nerve are shown in figure 2 in terms of density scans of radioautographs prepared from paper chromatograms of the chloroform extracts of these tissues. As in the mouse, the thiono isomer is converted into two principal chromatographically separated metabolite peaks, and the thiol isomer is converted into a single chromatographically separated metabolite peak. The same metabolites are produced *in vivo* as *in vitro*.

A comparison of the 2- and 4-hour curves (B-G curves, figure 2) shows that the metabolites increase as the thiono or thiol isomers decrease. The lower-chromatographing metabolite peak of the thiono isomer increases with time at the expense of the higher-chromatographing metabolite peak. This supports the data from mouse urine in which the lower-chromatographing metabolite peak was shown to be the final chloroform-partitioning metabolite or metabolites chromatographing as a single peak.

In contrast to the mouse tissues, in which only liver was appreciably active *in vitro* in metabolizing the isomers, all three tissues of the cockroach show marked activity. Although the gut is most active, both muscle and nerve clearly show metabolite peaks which are significantly greater than the correspondingly located peaks in the controls. The fact that smaller amounts of cockroach tissues than mouse tissues were used per flask in the *in vitro* experiments suggests that the oxidative enzymes responsible for metabolism are somewhat more generally located, at least quantitatively, in the cockroach than in the mouse.

Identity of the Animal Metabolites.—The identities of the metabolites from the *in vivo* and *in vitro* studies with mouse and cockroach tissues are suggested in part by a comparison of the average R_f values determined from 15 to 17 radioautographs for each isomer, with R_f values for a number of synthetic compounds prepared as possible metabolites (Fukuto *et al.* 1955). The data in table 3 show that the R_f values for the thionophosphate, thionophosphate sulfoxide (*O,O*-diethyl *O*-ethyl-2-sulfinylethyl phosphorothionate), and thiolphosphate are the same for both the compounds extracted from the animal tissues and the synthetic compounds within the limits of experimental error. On the basis of chromatography alone it is not possible in the case of the thiono isomer to separate and identify the thionophosphate sulfone (*O,O*-diethyl *O*-ethyl-2-sulfonylethyl phosphorothionate), the phosphate sulfoxide (*O,O*-diethyl *O*-ethyl-2-sulfinylethyl phosphate), or the phosphate sulfone (*O,O*-diethyl *O*-ethyl-2-sulfonylethyl phosphate); or in the case of the thiol isomer, the thiolphosphate sulfoxide (*O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate) and thiolphosphate sulfone (*O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate). The metabolite peak at the origin of the chromatogram in the case of each isomer could be a single metabolite or a combination of metabolites.

It is also difficult to interpret the upper regions of the chromatograms for both isomers from mouse and cockroach tissues (figures 1 and 2). It is apparent that these upper regions represent more than a single isomer. These double peaks may be artifacts due to the effects of other chloroform-soluble tissue extractives such as fat, which have a considerable influence on the movement and uniformity of the peaks on the chromatograms, or they may

be mixtures of the thiono and thiol isomers due to isomerization. There is some evidence that an additional metabolite, the phosphate (*O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphate) accounts in part for the 9- to 11-cm. peak in the curves for the thiono isomer. However, none of the compounds which might be present from theoretical considerations (Fukuto *et al.* 1955), except the thiono and thiol isomers and the phosphate, are found in this particular area of the chromatogram. On this basis it seems likely that these spots represent mixtures of the thiono and thiol isomers and the phosphate and not entirely unknown compounds. The cholinesterase inhibition data to be discussed below support this contention.

If the degradation of the two isomers and their metabolites is accomplished by enzymic breaking of the phosphorus-oxygen or phosphorus-sulfur bonds, as has been shown for di-*iso*propyl phosphorofluoridate (Mazur 1946, Mounter *et al.* 1953), para-oxon (Aldridge 1953), *O*-ethyl *N*-dimethyl phosphoroamidic fluoridate (Augustinsson & Heimbürger 1954), and *N,N'*-bis-dimethyl phosphorodiamidic fluoridate (Okinaka *et al.* 1954), then two phosphorus-containing ionic degradation products are possible for the thiono isomer and its metabolites, namely, *O,O*-diethyl thiophosphoric acid and *O,O*-diethyl phosphoric acid, whereas only the latter is possible for the thiol isomer, the phosphate, and their metabolites. It was not possible to separate these compounds from one another or from the chloroform-partitioning compounds by means of either of the paper-chromatographic techniques employed, and thus to establish their identities. However, their chloroform-water partitions and paper-chromatographic characteristics agree with those for pure *O,O*-diethyl thiophosphoric acid and *O,O*-diethyl phosphoric acid (table 5 and Fukuto *et al.* 1955), and it seems logical to assume from theoretical considerations that they or their salts or esters are the compounds involved.

Further evidence concerning the relationship of the various compounds isolated chromatographically from the chloroform extracts of mouse and roach tissues to the synthetic compounds was obtained by determination of the inhibition of house fly head cholinesterase. The data in table 4 show the anticholinesterase activities of the chromatographically separated compounds. Their concentrations were calculated from quantitative determinations of radioactivity on the chromatograms. Considering the many sources including various mouse and cockroach tissues and *in vivo* and *in vitro* experiments from which these compounds were obtained, the quantitative correlations between the various determinations for a single compound are remarkably good. A comparison of the $I_{50,s}$ for the isolated compounds and the synthetic compounds is given in table 5. In general there is good correlation between the relative activities in both groups. The very high anticholinesterase activity of the 9- to 11-cm. peak extracted from a chromatogram of the chloroform extract of the thiono isomer metabolized *in vivo* in roach gut, indicates that this peak contains considerable amounts of the phosphate, although the thiol

Table 4.—Inhibition of house fly head cholinesterase by the thiono and thiol isomers and their metabolites chromatographically separated from extracts of mouse or cockroach tissues.

Compound	R_f	Molar Concentration of Inhibitor	Per Cent Inhibition	Source of Isomer or Metabolite
		Thiono Isomer and Metabolites		
Thionophosphate sulfone or phosphate sulfoxide or sulfone	0.04	9.1×10^{-7}	21	Roach gut *in vivo*
		1.0×10^{-6}	57	Roach muscle *in vitro*
		2.8×10^{-6}	80	Roach gut *in vitro*
		4.1×10^{-6}	75	Mouse liver *in vitro*
		6.8×10^{-6}	91	Roach gut *in vitro*
Thionophosphate sulfoxide	0.24	1.0×10^{-6}	8	Roach gut *in vivo*
		1.1×10^{-6}	11	Roach muscle *in vitro*
		5.3×10^{-6}	13	Roach gut *in vitro*
		1.1×10^{-5}	55	Mouse liver *in vitro*
		1.2×10^{-5}	56	Roach gut *in vitro*
Thiono isomer	0.99	1.2×10^{-5}	47	Roach muscle *in vitro*
		1.8×10^{-5}	76	Roach gut *in vitro*
		2.4×10^{-5}	80	Mouse liver *in vitro*
Phosphate	0.72	2.0×10^{-7}	87	Roach gut *in vivo*
		Thiol Isomer and Metabolites		
Thiolphosphate sulfoxide or sulfone	0.06	1.2×10^{-6}	43	Roach thorax *in vivo*
		3.5×10^{-6}	89	Roach gut *in vivo*
		9.5×10^{-6}	97	Roach gut *in vitro*
		1.7×10^{-5}	97	Mouse liver *in vitro*
Thiol isomer	0.86	1.4×10^{-6}	57	Roach thorax *in vivo*
		3.1×10^{-6}	92	Roach gut *in vivo*
		1.0×10^{-5}	91	Roach gut *in vitro*
		3.4×10^{-5}	95	Mouse liver *in vitro*

Table 5.—Inhibition of house fly head cholinesterase by the thiono and thiol isomers and their metabolites prepared synthetically or separated chromatographically from extracts of mouse or cockroach tissues.

Synthetic Compound	I_{50} Synthetic Compound	I_{50} Extracted Compound	R_f Extracted Compound	Possible Identity of Compound Extracted from Animal Tissues
Thionophosphate	2.2×10^{-4}	1.2×10^{-5}	0.99	Thionophosphate
Thionophosphate sulfoxide	3.6×10^{-6}	1.0×10^{-5}	0.24	Thionophosphate sulfoxide
Thionophosphate sulfone	8.3×10^{-7}	1.8×10^{-6}	0.04	Thionophosphate sulfone or phosphate sulfoxide or sulfone
Phosphate	2.4×10^{-8}	$<2.0\times10^{-7}$ (87%)	0.72	Phosphate
Phosphate sulfoxide	1.1×10^{-6}	1.8×10^{-6}	0.04	Thionophosphate sulfone or phosphate sulfoxide or sulfone
Phosphate sulfone	1.2×10^{-7}	1.8×10^{-6}	0.04	Thionophosphate sulfone or phosphate sulfoxide or sulfone
Thiolphosphate	3.5×10^{-6}	1×10^{-6}	0.86	Thiolphosphate
Thiolphosphate sulfoxide	1.5×10^{-6}	1.3×10^{-6}	0.06	Thiolphosphate sulfoxide or sulfone
Thiolphosphate sulfone	6.0×10^{-7}	1.3×10^{-6}	0.06	Thiolphosphate sulfoxide or sulfone

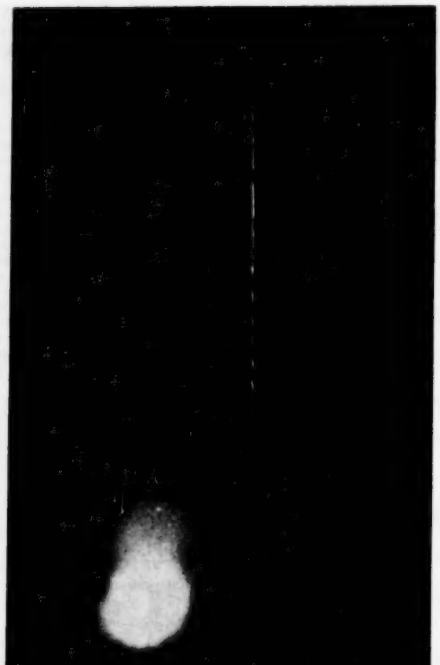

FIG. 3.—Radioautograph of propylene-glycol chromatogram of chloroform extract of cockroach thorax containing *in vivo* metabolized thiol isomer, showing two metabolite peaks at the origin.

isomer also could be present. The relatively low activity of the peak at the origin for the thiono-isomer metabolites, in comparison with the activity of the phosphate sulfone, suggests that this compound is not present in more than trace amounts.

Thus, on the basis of the evidence from paper chromatography and cholinesterase inhibition, it is possible to suggest the identity of the thiono and thiol isomers, the thionophosphate sulfoxide, and the phosphate. It is not possible to identify specifically the thionophosphate sulfone, phosphate sulfoxide, and phosphate sulfone, all of which could be present in the peak at the origin of the thiono-isomer chromatograms; nor is it possible to identify the thiolphosphate sulfoxide and sulfone which could be present in the peak at the origin of the thiol-isomer chromatograms. In a small number of chromatograms of the chloroform extracts of tissue-metabolized thiol isomer, two metabolite peaks were present at the origin (see figure 3). This infrequent peculiarity may be due to differences in impregnation of propylene-glycol on the paper (March *et al.* 1954). Although it is not possible to identify these peaks specifically, it seems logical, on the basis of the presence of the thionophosphate sulfoxide for the thiono isomer, that one of the two peaks is the thiolphosphate sulfoxide. The other, then, must be the thiolphosphate sulfone, this being the only other likely oxidative metabolite of the thiol isomer.

On the basis of anticholinesterase activity, it does not appear possible for the phosphate sulfone to be present in major quantity in the peak at the origin of the thiono-isomer chromatograms (see table 5). The relatively low toxicity of the phosphate sulfoxide to the mouse and to insects, in comparison with the thionophosphate sulfone (Fukuto *et al.* 1955), suggest that the phosphate sulfoxide, formed by oxidation of the thiono sulfur of the thionophosphate sulfoxide, is not the major final metabolite of the thiono isomer. It appears that the major pathway of oxidation is from the thionophosphate sulfoxide to the thionophosphate sulfone rather than to the phosphate sulfoxide. This is further suggested by the fact that this pathway of oxidation seems to be that for the thiolphosphate.

DISCUSSION.—The thiono and thiol isomers are rapidly metabolized, degraded, and eliminated in the mouse and cockroach by mechanisms in which three different biochemical systems are most likely involved. The biochemical changes resulting from the interaction of these systems in the animal are discussed below. Additional studies will be necessary to clarify and substantiate these hypotheses, in part, although they seem to fit the evidence developed in this study and in that reported in the preceding paper in this series (Fukuto *et al.* 1955).

From the toxicological standpoint the biochemical system of primary importance concerns the production of more toxic metabolites by the progressive oxidation of the mercapto sulfur of the ethylmercaptoethyl moiety, first to the sulfoxide and then to the sulfone. The thionophosphate and thiolphosphate sulfones are probably the final toxic metabolites for the thiono and thiol isomers, respectively, and the sulfoxide in each case is an intermediate metabolite in this process.

The second biochemical system of toxicological importance, which is concerned only with the thiono isomer and its metabolites noted above, involves the oxidation of the thiono sulfur to produce the phosphate and its sulfoxide and sulfone. These phosphate metabolites may be produced directly from the thiono isomer and its thiono metabolites by oxidation of the thiono sulfur, or they may be produced by the progressive oxidation of the ethylmercaptoethyl moiety of the phosphate. On the basis of previously reported results with thionophosphates such as parathion, in which oxidation of the thionophosphate to the phosphate has been shown to be responsible for *in vivo* toxicity and cholinesterase inhibition (Diggle & Gage 1951, Gage 1953, and Metcalf & March 1953), one might have expected that this same mechanism would be of primary importance in the case of the thiono isomer of Systox. The phosphate metabolites although present are not of primary importance in the animal. Their effective life is short apparently due to rapid degradation. This is undoubtedly related to their susceptibility to hydrolysis (Fukuto *et al.* 1955). Thus they are not found in quantity as principal metabolites.

The third biochemical system is of importance from the degradation standpoint and concerns the hydrolysis of the P-O- or P-S-bond of the ethylmercaptoethyl moiety, to form the ionic and nontoxic diethyl thiophosphoric acid or diethyl phosphoric acid and the respective alcohols. This system appears to be active toward both the thiono and thiol isomers and all their toxic metabolites. The final forms in which the acids or alcohols may be eliminated have not been elucidated, although it is possible that they are present as the salts

of the acids or as compounds formed with other alcohols or acids.

In gross aspects these biochemical mechanisms follow the same pattern in animals (white mouse and American cockroach) and plants (cotton, bean, and citrus; Metcalf *et al.* 1955a, and Fukuto *et al.* 1955). The major differences involved are concerned with rates of metabolism and degradation and with other considerations such as morphological differences. As one might expect, these rates are greater in the mammal than in the insect, and are greater in the insect than in the plant. From the standpoint of toxic residues in edible products, however, the important consideration involved here is that the routes of metabolism and the toxic metabolites formed are the same in both plants and animals. Thus there are no peculiar and highly toxic metabolites formed in the plant which the animal itself is not capable of producing and even degrading in a more rapid and efficient manner.

SUMMARY.—The thiono and thiol isomers of Systox are rapidly metabolized, degraded, and eliminated in the white mouse and American cockroach.

The principal route of elimination in the mouse is through the urine, 50 to 70 per cent of an orally administered dose being eliminated within 24 hours. Ninety per cent of the eliminated compounds are in the form of water-partitioning ionic degradation products. The remaining compounds in the urine are principally chloroform-partitioning toxic metabolites, only traces being eliminated as the unchanged isomers. The liver is the principal organ of metabolism and degradation.

Metabolism, degradation, and elimination are somewhat slower in the cockroach than in the mouse, but in gross aspects they are the same. The foregut exhibits a marked selective absorption of the isomers, and the gut is the principal avenue of elimination. Although the gut appears to be the most active tissue in the role of metabolism and degradation, other tissues such as nerve and muscle are more active in the roach than in the mouse.

The principal pathway of metabolism to toxic metabolites for both isomers is the oxidation of the mercapto sulfur of the ethylmercaptoethyl moiety to the sulfoxide and sulfone. A secondary pathway in the case of the thiono isomer involves the oxidation of the thiono sulfur to produce the phosphate and its sulfoxide and sulfone. Both isomers and their toxic metabolites are degraded by hydrolysis of the P-O- or P-S-bond to form the alcohol and acid.

The routes of metabolism and the toxic metabolites formed are the same in plants and animals. Thus toxic residues in edible products present no peculiar hazard as a result of biochemical activity in the plant.

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The Nature and Significance of Systox Residues in Plant Materials[1,2]

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The three previous papers of this series (Metcalf *et al.* 1954b, Fukuto *et al.* 1955, March *et al.* 1955) have presented information regarding the behavior of Systox[3] as a systemic insecticide, and concerning its biochemical transformation in plants and animals. It is the purpose of this report to assess the effects of these developments upon the estimation and significance of Systox residues in edible plant materials.

MATERIALS AND METHODS.—The investigations to be described were carried out largely by the use of P^{32} radiotracers of the two Systox isomers *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate (thiono isomer) and *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate (thiol isomer), and by paper chromatography as described by Fukuto & Metcalf (1954) and by March *et al.* (1954).

Processing Samples for Analysis.—Surface residues on leaves and fruit of orange, apple, walnut, and pear were determined by stripping these in acetone solution to a volume of 250 ml., concentrating in a Kuderna-Danish evaporator to 5 ml., and evaporating 1-ml. aliquots in planchets for radioassay.

Chloroform was used as the solvent in all the extractions of plant materials for radioassay. It has been shown that this solvent is very effective in extracting not only the thiono and thiol isomers but also the toxic metabolites formed from these in plants (Heath *et al.* 1953, Metcalf *et al.* 1954b).

Apples and Pears.—Stripped replicates of 10 fruits, weighing 350–650 g., were thoroughly minced in 500 ml. of chloroform. The mince was sealed in a stainless-steel can and tumbled for 1 hour, filtered, washed twice with chloroform, and the extract and washings separated. The total chloroform extract or a 100- to 200-ml. aliquot was concentrated in a Kuderna-Danish evaporator to a volume of 5 ml., and 1-ml. aliquots were evaporated for radioassay. For determination of residues in pulp uncontaminated by peel, the 10 fruit were carefully split, and three scoops of pulp removed from the center of each (away from the peel), combined, minced, and extracted as above. Fortified samples containing known amounts of radioactive Systox were processed in the same way and the recovery data presented in table 1 were obtained.

Table 1.—Recovery data on samples fortified with P^{32}-labeled technical Systox.

Sample Wt. g.	Added		Recovered	
	γ	p.p.m.	γ	Per Cent
		Potato Pulp		
995.6	99.6	0.1	89	89
737.6	73.8	1.0	73.0	99
		Orange Pulp		
500.7	5	0.01	4.8	96
511.7	15.4	0.03	16	104
668.5	67	0.1	69	103
643.4	194	0.3	189	97
637	637	1.0	682	107
		Walnut Meat		
48	4.8	0.1	3.9	81
		Apple Pulp		
811	8.11	0.01	7.30	91
896	26.9	0.03	26.9	100

Oranges.—Replicates of 10 stripped oranges, averaging about 1000 g., were carefully peeled to avoid any possibility of contaminating the pulp. The peel and pulp were separately minced, extracted, concentrated, and radioassayed as described above. Fortified samples were processed in the same way. Recovery data are given in table 1.

Walnuts.—Replicates of 20 stripped walnut fruits weighing from 750 to 950 g., were carefully dried at 43.5° C. (110° F.) for 5 days to bring the moisture content to a suitable level, as is the commercial practice. The nuts were then shelled and cracked, and the total meats, averaging about 100 g., minced in a Waring blendor with 200 ml. chloroform, tumbled for 1 hour, filtered, and concentrated, as far as possible, in a Kuderna-Danish evaporator. Because of the large amounts of oil present, these extracts could not be evaporated for counting. Therefore, the chloroform-oil concentrate was shaken with 100 ml. of acetonitrile for 1 hour and separated, and the chloroform-oil layer further extracted with two 25-ml. portions of acetonitrile. The combined acetonitrile layers were concentrated to 3 ml. in the Kuderna-Danish evaporator, and 1-ml. aliquots evaporated for radioassay.

Potatoes.—Replicates averaging about 1000 g. each were minced in 750 ml. of chloroform and extracted as described above. The total chloroform layer was separated, filtered, and the pulp washed twice with chloroform. The combined chloroform layers were concentrated to 5 ml., and 1-ml. aliquots were evaporated for radioassay.

Sugar Beets.—Replicates of the roots, averaging 460 g. each, were homogenized in chloroform in a Waring blendor. The chloroform layer was separated, aliquots concentrated to 5 ml., and 1-ml. aliquots evaporated for assay.

The technics for field application of radioactive Systox have been previously described (Metcalf *et al.* 1954a).

Radioassay.—The radioactivity of the samples in 1-inch planchets was determined by counting with an end-

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[3] Trade-mark, Chemagro Corporation, New York, N. Y.

window Geiger tube of 1.4 mg./sq. cm. thickness. Each sample was counted for at least 600 seconds to ensure statistical reliability, and the counts were corrected for background. Samples having less than 0.03 c.p.s. greater than background were considered as possessing no activity, since this value represents the standard deviation of the average background. Duplicate determinations were made on each sample, and in the very infrequent cases of disagreement a third sample was counted. The values reported in tables 2 and 3 are averages of the two determinations corrected for the radioactive decay of P^{32} (half-life, 14.3 days), using curves prepared by counting known weights of P^{32}-Systox at intervals. The data are reported as micrograms of Systox isomers (molecular weight 258), and thus represent the maximum residues present, since the active metabolites have molecular weights very close to that of Systox.

Partition of the radioactivity in the plant materials between water and chloroform was accomplished by centrifuging an aliquot of homogenate to remove particulate material, separating 3 ml. and shaking this for 1 minute with an equal volume of chloroform. The layers were separated and the radioactivity was determined per unit volume.

Cholinesterase Assay.—Systox residues in oranges and pears were also evaluated by the cholinesterase technic of Hensel *et al.* (1954). This method was used without modification, except that a preparation of purified human-plasma cholinesterase, concentrated 88 times, was used at the rate of 2 mg. per ml. of 0.9 per cent NaCl, instead of human plasma. Duplicate determinations were made with all samples and the results were averaged. In most cases involving high activity, serial dilutions were made and the results were calculated from the optimum portion of the inhibition curve. The oranges were washed, peeled, and thoroughly minced to form juice. The pears were washed in soap and water and minced in an equal weight of water in a Waring blendor. The residues in leaf tissues were determined by this technic by homogenizing in 10 parts of water by weight in a Potter-Elvejhem homogenizer.

Table 2.—Comparison of Systox residues from duplicate samples by radioactive and enzymatic methods.

Substrate Treatment	Days After Application	P.P.M. Active Esters			
			ChE		
				Thiolphosphate	
		Radiotracer	Tech. Systox	Sulfoxide	Sulfone
Orange juice	30	0.24	3.5	0.80	0.25
Thiono isomer	37	0.35	1.25	0.25	0.09
	44	0.63	7.7	1.80	0.56
Thiol isomer	30	1.42	19	4.2	1.3
	37	2.65	44	10.0	3.0
Apple pulp	1	0.21	1.34	0.30	0.10
Thiono-thiol	2	0.20	3.9	0.90	0.28
mixture (1:2)	5	0.25	2.04	0.45	0.14
	10	0.54	1.6	0.36	0.11
Bean foliage					
Thiono isomer	3	268	420	95	28
Thiol isomer	3	4440	27000	6500	2000
Cotton foliage					
Thiono isomer	3	108	460	105	33
	8	161	630	145	46
	11	37	200	64	20
	24	93	175	40	12
Thiol isomer	8	124	1000	230	70
	11	104	480	110	34
	24	3990	10000	2300	700

Table 3.—Systox spray residues.

Dosage, Oz. Tech. Systox/ 100 Gal.	Days Between Application and Harvest	No. of Samples[a]	Method	Substrate	Residue, P.P.M. (Average)
			Valencia Oranges		
1	14	8	Enzyme	Juice	<0.1[b]
1	28	8	Enzyme	Juice	<0.1[b]
4	14	8	Enzyme	Juice	<0.1[b]
4	28	8	Enzyme	Juice	<0.1[b]
4	14	20	Radiotracer	Juice	0.007±0.0053
4	14	20	Radiotracer	Peel	0.22±0.11
4	14	20	Radiotracer	Fruit Surface	0.055±0.014
4	14	20	Radiotracer	Leaf Surface	2.20±0.92
4	14	20	Radiotarcer	Leaf Interior	2.44±1.3
4	28	20	Radiotracer	Juice	0.013±0.0054
4	28	20	Radiotracer	Peel	0.24±0.085
4	28	20	Radiotracer	Fruit Surface	0.024±0.009
4	28	20	Radiotracer	Leaf Surface	2.25±1.1
4	28	20	Radiotracer	Leaf Interior	1.04±0.57
4	31	10	Radiotracer	Juice	0.020±0.0059
4	31	10	Radiotracer	Peel	0.152±0.072
4	31	10	Radiotracer	Fruit Surface	0.027±0.013
			Apples		
2	19	10	Radiotracer	Peel & Pulp	0.084±0.065
2	19	10	Radiotracer	Pulp	0.036±0.022
2	19	10	Radiotracer	Leaf Surface	0.043±0.02
2	30	10	Radiotracer	Peel & Pulp	0.0068±0.0035
2	30	10	Radiotracer	Pulp	0.0027±0.00099
2	30	10	Radiotracer	Leaf Surface	0.0053±0.0029
			Pears		
3	21[c]	6	Enzyme	Peel & Pulp	<0.1[b]
3	14[d]	6	Enzyme	Peel & Pulp	0.14[b]
3	14[e]	6	Enzyme	Peel & Pulp	0.1[b]
2	30	4	Radiotracer	Peel & Pulp	0.056
2	30	4	Radiotracer	Pulp	0.030
2	30	4	Radiotracer	Fruit Surface	0.017
			Walnuts		
4	14	20	Radiotracer	Hull	0.013
4	14	20	Radiotracer	Leaf Surface	0.05
4	30	20	Radiotracer	Meats	0.0095±0.0085
4	30	20	Radiotracer	Leaf Surface	0.033±0.0099
			Potatoes		
4	19	8	Radiotracer	Tubers	0.016
4	19	8	Radiotracer	Leaf	12.37
2	19	6	Radiotracer	Tubers	0.105
2	19	6	Radiotracer	Leaf	0.74
2	30	6	Radiotracer	Tubers	0.068
2	30	6	Radiotracer	Leaf	0.94
4	33	8	Radiotracer	Tubers	0.052
4	33	8	Radiotracer	Leaf	.388
			Sugar Beets		
4	30	4	Radiotracer	Root	0.000
4	30	4	Radiotracer	Leaf	11.90
4	30	4	Radiotracer	Root	0.130
4	30	4	Radiotracer	Leaf	3.60

[a] Two replicates.
[b] Corrected to p.p.m. of thiolphosphate sulfoxide.
[c] One application.
[d] Two applications.
[e] Three applications.

RESULTS.—*Nature of Metabolites in Edible Produce.*—Paper-chromatographic studies using both the silicone and the glycol system have been carried out on the metabolites of the thiol and thiono isomers in orange, walnut, and apple, at intervals of 7 to 30 days after application. These studies were made on fruits treated with dosages of radioactive Systox isomers about 30 times those normally employed in pest control, in order to obtain sufficient quantities of radioactive products in the edible materials to permit accurate quantitative and qualitative analysis.

The data obtained show that the Systox isomers are metabolized in the fruits in the same manner as has been demonstrated in the leaves of bean, cotton, and lemon

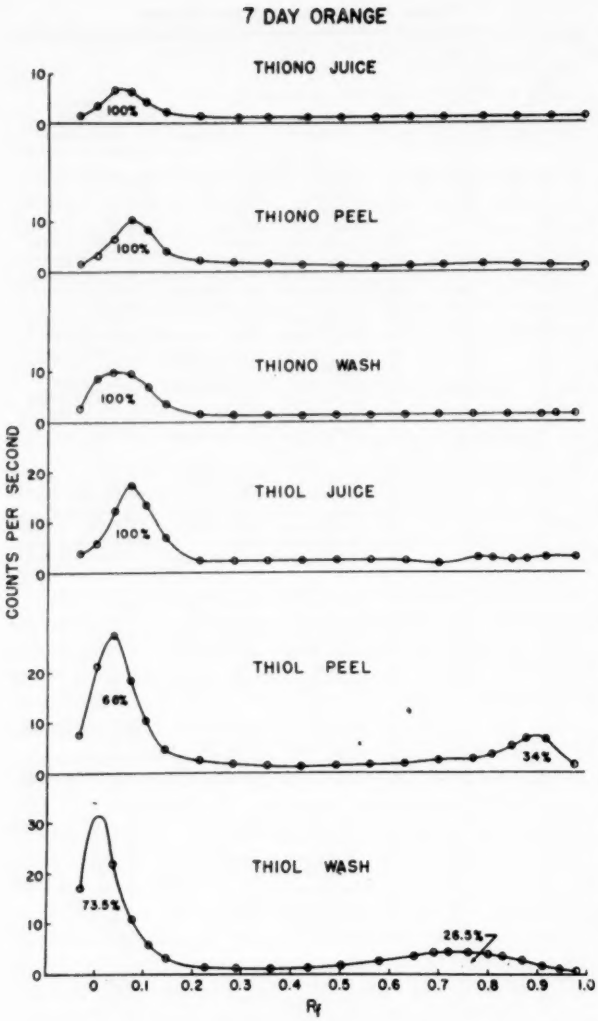

FIG. 1.—Chromatograms on propylene-glycol paper, showing thiono and thiol isomers in chloroform extracts of juice, peel, and surface wash of Valencia oranges 7 days after the fruit had been dipped in concentrated solutions of the P^{32}-labeled isomers.

(Metcalf *et al.* 1954b, Fukuto *et al.* 1955). This is graphically shown in figure 1, which demonstrates that 7 days after treatment with the thiono isomer, only traces of this isomer were present in the surface wash of Valencia oranges or in the peel or juice, all the radioactivity being present at a location on the chromatogram corresponding to *O,O*-diethyl *O*-ethyl-2-sulfinylethyl phosphate (phosphate sulfoxide) and *O,O*-diethyl *O*-ethyl-2-sulfonylethyl phosphorothionate (thionophosphate sulfone).

In oranges treated with the thiol isomer, considerable amounts of this isomer were found in the wash and in the peel after 5, 7, and 16 days, but two-thirds or more of the radioactivity was present at a location corresponding to *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate (thiolphosphate sulfoxide), and *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate (thiolphosphate sulfone), and these materials comprised 100 per cent of the radioactivity in the juice.

A similar experiment was carried out with a 65:35 mixture of thiono and thiol isomers for treating orange, apple, and walnut. In this experiment neither of the starting materials was found in orange juice or peel, apple peel or pulp, or walnut meat in periods of from 7 to 30 days after treatment, and the chromatographically isolated radioactivity always corresponded to the oxidative metabolites, as previously mentioned. However, the surface washes from orange, apple, and walnut at the 7- and 15-day period showed a considerable proportion of thiono isomer and traces of thiol isomer, although after 30 days only trace amounts of thiono isomer could be found.

The indications that the chemical nature of the surface washes from thiono and thiol isomer-treated fruits rapidly changes by exposure to light and air aroused interest in the effects of such exposure upon the isomers, uncomplicated by biological substrates. This was observed by adding 5λ of the purified radioactive isomers into petri-dish halves. The compounds were distributed over the glass surfaces by adding 1 ml. acetone, swirling, and allowing the acetone to evaporate. The uncovered dishes were then exposed to midday sunlight for varying periods. The temperature of the dishes during exposure ranged from 30 to 40° C. Aliquots of material were removed from the dishes at intervals and chromatographed, with the results shown in figure 2. These indicate a surprisingly rapid conversion of the Systox isomers into compounds which appear to be chromatographically similar to those found within the plant tissues. The transformations occurred rapidly, and after 3 hours only traces of the starting materials remained. It is obvious that the chemical changes must influence the systemic behavior of the compounds, both from the standpoint of persistence of surface residues and penetration into the interior of the plant.

Rate of Destruction of Metabolites.—The chloroform/water partition coefficients of the radioactive extracts from Systox-treated plant materials are of value in distinguishing between the biologically active thiono and thiol isomers and their oxidation products, which partition about 98 per cent in the chloroform phase; and the inactive hydrolysis products such as *O,O*-diethyl phosphoric acid and *O,O*-diethyl thiophosphoric acid, which partition about 98 per cent in the water phase (Fukuto *et al.* 1955). The rate of formation of these decomposition products is influenced by the hydrolysis constants of the esters (Fukuto *et al.* 1955) and by the possible action of esterase enzymes within the plant. The over-all rates of decomposition in representative plant tissues are given in figure 3 where the $HCCl_3/H_2O$ partition coefficients are plotted against time after treatment. It will be seen that decomposition of the biologically active compounds begins almost immediately upon their entrance and/or formation in the plant material. The rates of decomposition of the thiol isomer metabolites are only about one-half those of the thiono isomer metabolites, and this is to be expected from the relative stabilities of the important active metabolites, as discussed by Fukuto *et al.* (1955). From the data in figure 3 it appears that the

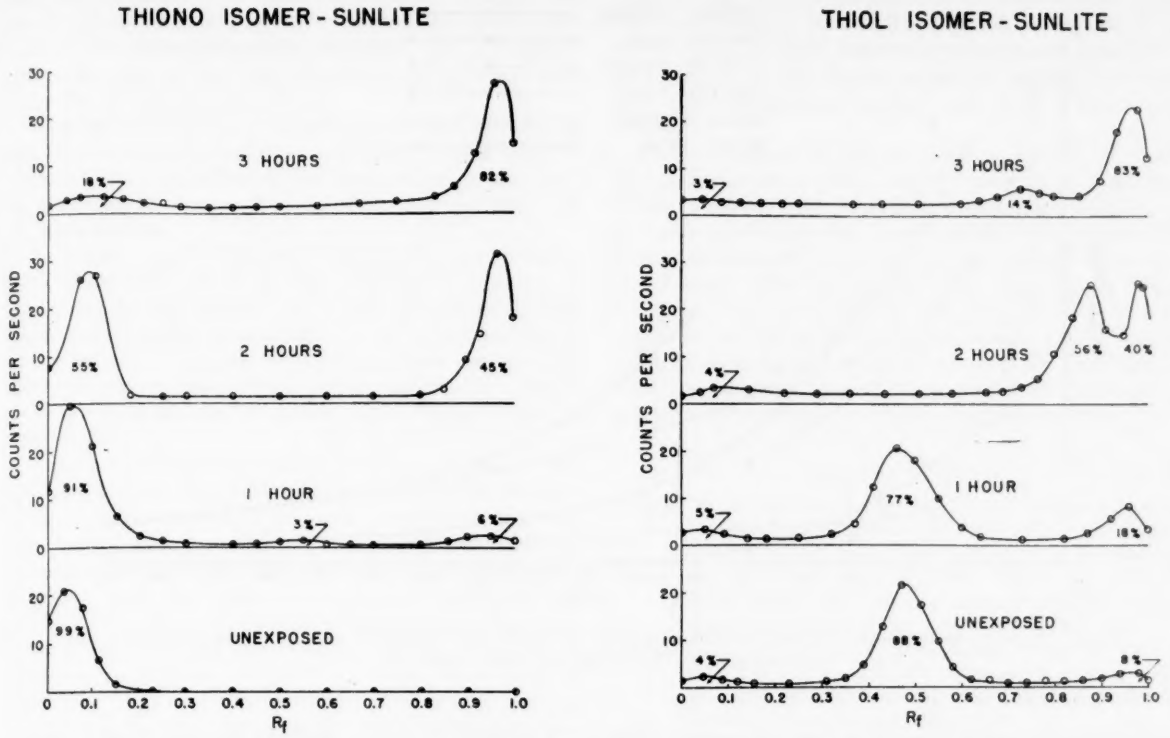

FIG. 2.—Chromatograms on silicone-impregnated paper, showing effect of exposure of P^{32}-labeled thiono and thiol isomers to direct sunlight for different periods.

approximate times in days for decomposition of the active metabolites are:

	D_{50}	D_{90}
Thiol isomer		
Leaves	18	38
Orange juice	30	61
Thiono isomer		
Leaves	8	16
Orange juice	20	40

Application to Cholinesterase Assay.—The cholinesterase technic of Hensel *et al.* (1954) has been used extensively for the quantitative determination of Systox residues in plant materials. However, the magnitude of the values obtained depends upon the anticholinesterase activity of the technical Systox which is used to prepare the standard curve. It has been shown that the thiono isomer which comprises two-thirds of the technical material is a poor cholinesterase inhibitor (Fukuto *et al.* 1955); therefore, the anticholinesterase action is primarily a result of the thiol isomer content. However, since neither isomer has been found to persist in appreciable amounts in plant tissues, it is clear that the accuracy of the cholinesterase technic is dependent upon the relative anticholinesterase activities of the active plant metabolites (Fukuto *et al.* 1955). It has been shown previously that the thiol isomer metabolites accumulate in plants from 5 to 10 times as rapidly as the thiono isomer metabolites (Metcalf *et al.* 1954b), and that the former are considerably more persistent, as discussed above. Therefore, it would seem that a more realistic and accurate picture of toxic residues might be obtained by referring the cholinesterase inhibition of plant samples to standard curves based on the thiolphosphate sulfoxide and thiolphosphate sulfone which are apparently the important toxic metabolites, as shown in figure 4.

In order to gain insight into the actual magnitude of the Systox residues, comparisons have been made of the residues in leaves and fruits as determined both by the cholinesterase technic and by the radiotracer technic, using aliquots of the same treated samples for the determinations. The plant material used was treated with heavy dosages many times those used in commercial practice, in order to ensure the presence of residues in large enough amounts for accurate determination. In all cases, the radioactivity was calculated on the basis of the chloroform extractives, which appear to comprise the biological active esters, as shown previously (Metcalf *et al.* 1954a). The results of the comparisons are given in table 2. It will be noted that the residues obtained by the cholinesterase technic based on technical Systox, are consistently from 3 to 10 or more times greater than those from radioassay. From reference to figure 4 it is clear that the cholinesterase inhibition from the thiolphosphate sulfoxide is about 5 times, and that from the thiolphosphate sulfone about 10 times, that of technical Systox, and it is shown in table 2 that the use of the standard curves with these materials (Fig. 4), which in effect would reduce these residue values to about 0.1 to 0.2 of the original, would provide values which fall much

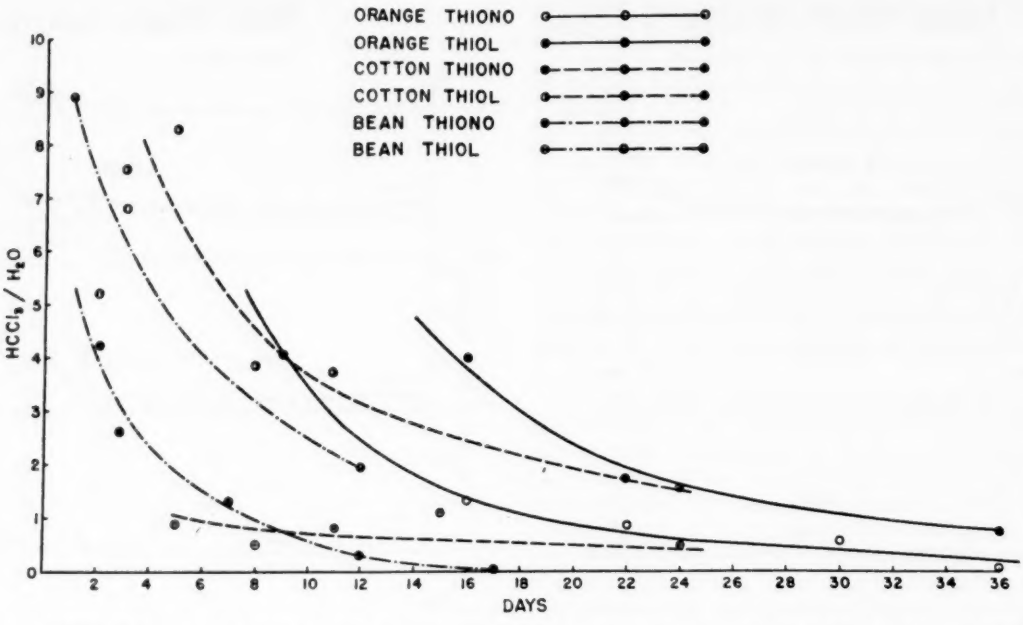

FIG. 3.—Rate of decomposition of thiol and thiono isomer metabolites in citrus pulp and in bean and cotton leaves, as measured by $HCCl_3/H_2O$ partition coefficients.

better into line with the figures from radioassay. The metabolic formation of these more active anticholinesterase oxidation products is responsible for the apparent increase in Systox residues (as measured by the cholinesterase method) which has sometimes occurred at various periods after treatment.

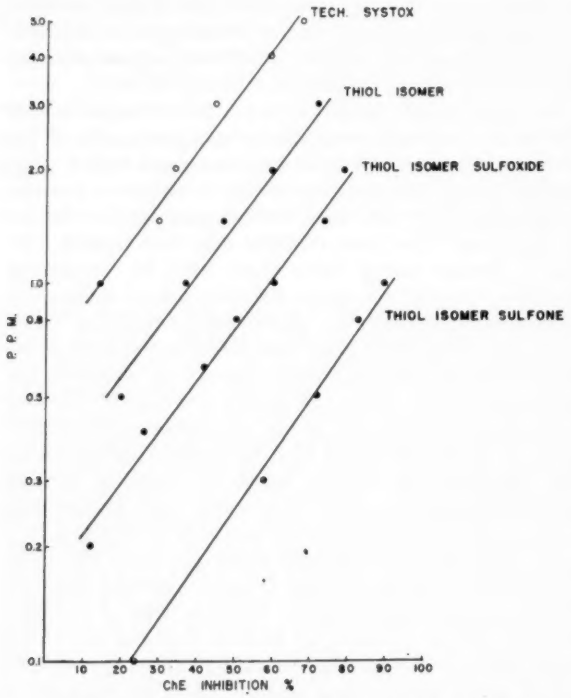

FIG. 4.—Curves showing relative inhibition of human plasma cholinesterase, determined by the electrometric technic.

Estimation in Systox Residues Resulting from Commercial-type Spray Application.—Estimation of the actual residues resulting from the practical application of Systox sprays is of great importance in determining the safety of such treatments to the consumer of the treated produce. Because of its sensitivity, the assay with radioactive Systox was selected as the most accurate and reliable method available. Radioactive Systox consisting of 1 part of a 65:35 mixture of thiono and thiol isomers and 3 parts of polyglycol emulsifier,[4] as used in the commercial insecticide, was applied to several food crops at the dilutions and rates of application suggested for commercial practice (Metcalf *et al.* 1954a). The harvest-time residues which resulted are presented in table 3, together with several determinations by the enzyme method. It will be seen that the two methods gave results which were in substantial agreement, although the residues found were below the limit of detection of the enzyme assay. The average residue values 4 weeks after application were as follows: orange juice, 0.013 p.p.m.; total apple peel and pulp, 0.007 p.p.m.; walnut meats, 0.0095 p.p.m.; pears, 0.03 p.p.m.; and potatoes, 0.96 p.p.m. The maximum amounts found were: orange juice, 0.048 p.p.m.; apple, 0.011 p.p.m.; walnuts, 0.043 p.p.m.; and potatoes, 0.105 p.p.m.

SUMMARY AND CONCLUSIONS.—The metabolism of the thiono and thiol isomers of Systox in orange, apple, and walnut has been shown to take place in the same fashion as previously described for leaves of various plants (Metcalf *et al.* 1954b, Fukuto *et al.* 1955). Within 1 or 2 weeks after the application of either of the isomers, almost no trace of the starting materials could be found in the plant tissues, and paper-chromatographic technics

[4] *Thiosolve 8139.*

showed the presence of oxidative products of which the major constituents appear to be the thiolphosphate sulfoxide, the thionophosphate sulfoxide, the phosphate sulfoxide, and/or the thionophosphate sulfone, and perhaps the thiolphosphate sulfone.

The action of air and sunlight on surface residues of the Systox isomers also has a rapid effect, and appears to promote their oxidation in the same sequence as found *in vitro* with hydrogen peroxide (Fukuto *et al.* 1955) and in plant tissues.

In addition to activation in the plant tissues to oxidative metabolites, these are subsequently hydrolyzed to nontoxic, diethyl phosphoric acids and alcohols. The thiol isomer metabolites persist in leaf and fruit tissues about twice as long as the thiono isomer metabolites. The respective half-lives in cotton leaves and orange pulp are thiol isomer, 18 and 30 days; thiono isomer, 8 and 20 days.

A consideration of the various factors as outlined in the previous parts of this study (Metcalf *et al.* 1954b, Fukuto *et al.* 1955, March *et al.* 1955) leads to the conclusion that the thiolphosphate sulfoxide and the thiolphosphate sulfone are probably the principal toxic plant metabolites resulting from the pesticidal application of Systox, especially since the thiol isomer and its metabolites have been shown to accumulate in treated plants from 5 to 10 times as rapidly as the products from equal rates of treatment with the thiono isomer. The thiolphosphate sulfoxide is about 5 times and the thiolphosphate sulfone about 10 times more active as cholinesterase inhibitors than technical Systox. Therefore, residues obtained by the cholinesterase technic (Hensel *et al.* 1954) would appear to be approximately 5 to 10 times too large, and this has been demonstrated by comparisons of the residues in plant samples treated with radioactive Systox, as determined by radioassay and by cholinesterase assay.

It is not considered that the presence of the toxic oxidative metabolites in edible produce should provide any additional concern in assessing the residue hazards resulting from Systox application. March *et al.* (1955) have shown that the process of metabolism of the Systox isomers in the mammal is identical with that in plants. The animal organism is therefore exposed to essentially the same toxic processes as the plant, regardless of whether the isomers are ingested in their original or in their metabolized forms. The hydrolysis of the toxic metabolites in plant materials to nontoxic phosphoric acid derivatives is a further safeguard against the retention or accumulation of the toxic esters over a long period of time.

The estimation of the maximum amounts of toxic Systox metabolites which may be present in plant materials treated with standard commercial dosages of Systox is also reassuring. Investigations with radiotracer Systox have shown that the average residue values at 2 and 4 weeks after application were substantially below 0.1 p.p.m. and generally in the range of 0.01 to 0.03 p.p.m. for orange juice, total apple peel and pulp, walnut meat, pear, and potato. These amounts are generally of such low magnitude that they could not have been precisely determined by other than tracer technics.

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Respiratory Response of Avocado Fruits to Fumigation Effective Against the Eggs and Larvae of Fruit Flies[1]

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The possibility of various fruit flies becoming established in California has been the reason for initiating a program of research on the effectiveness of various sterilization practices against these insects and on the subsequent effect of these practices upon the physiology of the plant material. Although vapor heat sterilization is effective against the insects, chemical fumigation appears more practicable from the standpoint of fruit tolerance (Lindgren & Sinclair 1951; California State Legislature, "Third Special Report on the Control of the Oriental Fruit Fly (*Dacus dorsalis*) in the Hawaiian Islands," 1953; Sinclair & Lindgren 1955). Lindgren & Sinclair

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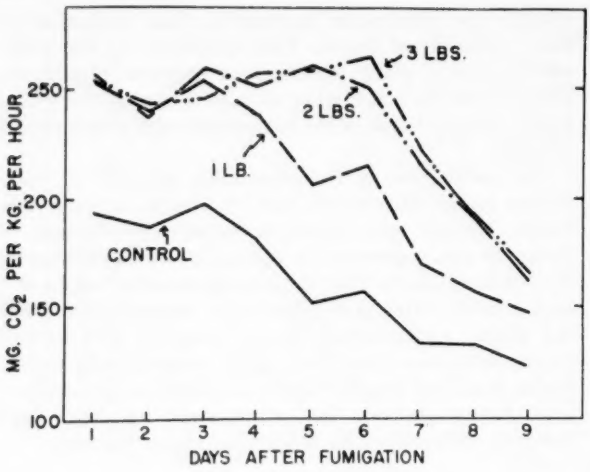

FIG. 1.—Respiration rate (mg. CO_2 per kg. per hour) of postclimacteric avocado fruits stored at 68°F. after fumigation at 70° F. with 1, 2, and 3 pounds of ethylene dibromide per 1000 cubic feet for 2 hours.

(1951) observed no injury to avocado fruits after fumigation with 1, 2, or 3 pounds per cubic foot of ethylene dibromide and ethylene chlorobromide for 2 hours at 70° F.; however, these fruits failed to tolerate methyl bromide fumigation at a dosage of 2 pounds per 1000 cubic feet for 4 hours. Subsequently, the fumigation of certain varieties with 2 and 3 pounds of ethylene dibromide per 1000 cubic feet for 2 hours was found to result in surface discoloration (Lindgren *et al.* 1955).

It became apparent during the pursuit of this project that a more objective criterion was needed to evaluate the physiological response of the fruit to fumigation, one which would make it unnecessary to wait for the injury to develop during storage. Accordingly, a study of the respiratory activity of avocado and citrus fruits following various fumigation treatments was undertaken. Results obtained with avocado fruits are presented here.

MATERIALS AND METHODS.—The avocado fruits of the Fuerte variety used in this study were freshly picked, selected fruits, except for the postclimacteric fruits used in one experiment, which were commercially packed fruits of unknown history. Except for the postclimacteric fruits, all fruits were picked, fumigated, and set up in respiratory chambers at 68°F. the same day. In all cases four replications of each treatment were set up for respiratory rate determinations.

The fumigation was carried out at a temperature of 70°F. and a relative humidity of 80 per cent or above, with continuous air circulation, in a 100-cubic-foot gastight metal fumatorium. Dosages are expressed as pounds of fumigant (ethylene dibromide, ethylene chlorobromide, or methyl bromide) per 1000 cubic feet of chamber space. The duration of fumigation was 2 hours for all treatments except for the 1½-pound dosage of ethylene dibromide, for which a 4-hour exposure was used. Respiration rate determinations were made twice daily, starting the next morning after fumigation and continuing throughout the storage life of the fruit. The Claypool & Keefer (1942) colorimetric method was used to determine the rate of carbon dioxide production. The capillaries used to meter the air through the respiratory chambers were calibrated frequently to insure accuracy. During each experiment and when each was terminated the fruits were rated for softness and the ratings were given numerical values as follows: firm (0), softening (25), ideal eating stage (50), soft (75), and inedible (100). A softening index was calculated by multiplying the number of fruit in each category by the respective value, summating the products, and dividing by the total number of fruits in the sample.

RESULTS.—Avocado fruits fumigated with 1, 2, and 3 pounds of ethylene dibromide per 1000 cubic feet for 2 hours, after the climacteric peak of respiration, showed a significant stimulation of approximately 25 per cent in respiration rate over comparable untreated fruit (Fig. 1). During the first 3 days the fruits showed no differential response to the various treatments. However, after 3 days the respiration rate of the fruit fumigated with 1 pound of ethylene dibromide per 1000 cubic feet began to decline, while that of the other treatments remained at about the same level until the sixth day and then declined rapidly. Some surface discoloration was noted on fruits receiving the 2- and 3-pound treatments, these fruits also softening more rapidly than those of the other treatments.

Freshly harvested preclimacteric avocado fruits were stimulated even more than postclimacteric fruit, as illustrated in figure 2. The preclimacteric fruits fumigated with 2 and 3 pounds for 2 hours and 1½ pounds for 4 hours were stimulated to about the same level (250 mg. CO_2 per kg. per hour) and followed about the same pattern as the postclimacteric fruits receiving the 2- and 3-pound dosages for 2 hours (Fig. 1)—that is, they showed no climacteric rise. Fruit fumigated with 1 pound for 2 hours respired at an initial rate about 50 per cent higher than untreated fruit, but went through a normal climacteric rise to a peak, after 5 days, of nearly the same magnitude as the peak reached by the control fruit after 8 days. The average softness indexes for the fruits 6 days after fumigation at various dosages for 2 hours are as follows: control.

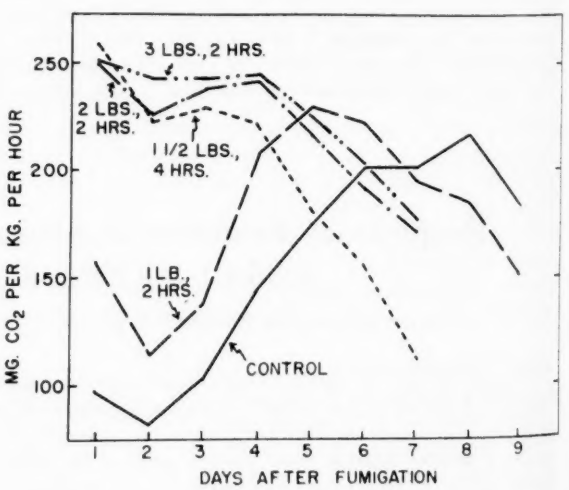

FIG. 2.—Respiration rate (mg. CO_2 per kg. per hour) of preclimacteric avocado fruits stored at 68° F. after fumigation at 70° F. with various dosages of ethylene dibromide per 1000 cubic feet for different periods.

33; at 1 pound, 45; at 2 pounds, 49; at 3 pounds, 53. At a dosage of 1½ pounds for 4 hours, the index was 50.

Cumulative carbon dioxide has been used by many investigators to evaluate respiratory responses more critically. Accordingly, the cumulative carbon dioxide production of the preclimacteric fruits receiving the various ethylene dibromide treatments was calculated by multiplying the average daily respiration rate (mg. CO_2 per kg. per hour) by 24 and summating the values expressed as grams of carbon dioxide per kilogram of fruit (Fig. 3). In figure 3 very little differences will be noted between the three higher dosages, while a definite segregation occurs between the three higher dosages, the 1-pound 2-hour treatment and the control. The cumulative carbon dioxide production graph has limitations in that the climacteric rise and peak are obscured. In this form, however, the data do portray more explicitly the significant differences in the respiratory response of avocado fruits to ethylene dibromide fumigation.

Ethylene chlorobromide fumigation at dosages of 1 and 3 pounds for 2 hours resulted in about the same relative initial stimulation (Fig. 4) as the same dosages of ethylene dibromide (Fig. 2). However, the rate for fruit fumigated with the 3-pound dosage declined and appeared to go through a climacteric rise. Again the treated samples reached slightly higher climacteric peaks than the untreated samples and reached it sooner. The average softness indexes after 6 days were 54 for the control, 58 for those receiving the 1-pound dosage, and 62 for those receiving the 3-pound dosage.

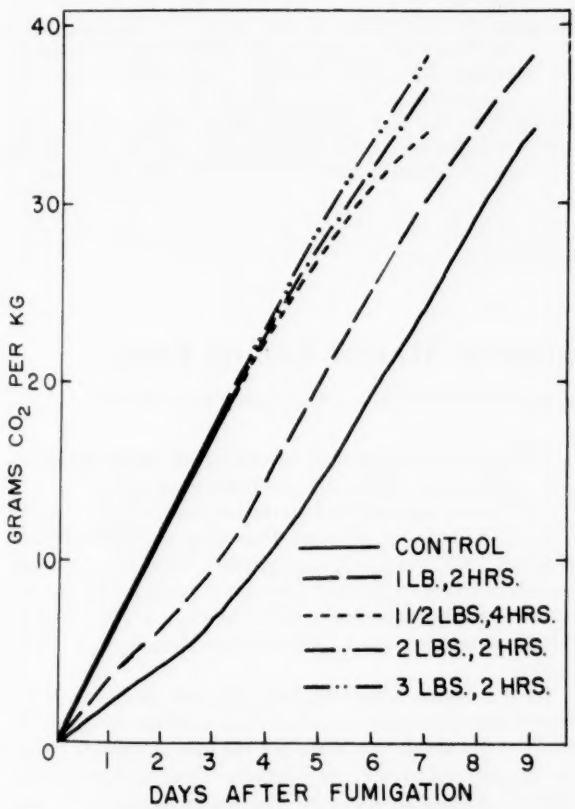

FIG. 3.—Respiration rate expressed as the cumulative carbon dioxide production (grams per kg.) of preclimacteric avocado fruits stored at 68° F. after fumigation at 70° F. with various dosages of ethylene dibromide per 1000 cubic feet for different periods.

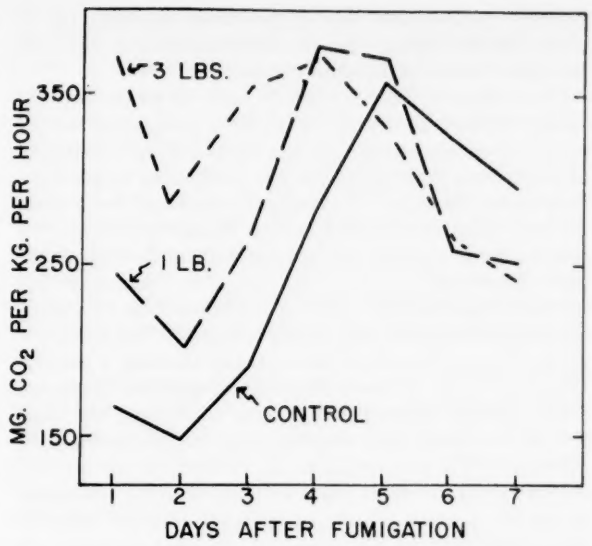

FIG. 4.—Respiration rate (mg. CO_2 per kg. per hour) of preclimacteric avocado fruits stored at 68° F. after fumigation at 70° F. with 1 and 3 pounds of ethylene chlorobromide per 1000 cubic feet for 2 hours.

Avocado fruits fumigated with 2 and 4 pounds of methyl bromide (Fig. 5) showed higher initial respiration rates than untreated fruit. Both treatments appeared to have a climacteric rise to about the same maximum as the untreated fruits; however, the 4-pound dosage appeared to delay the climacteric about a day. This delay was also exemplified by the average softness indexes after 6 days, which were 40 for the controls, 50 for fruits receiving the

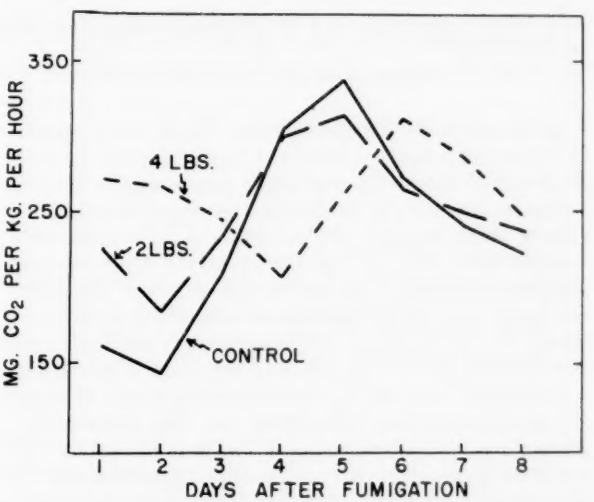

FIG. 5.—Respiration rate (mg. CO_2 per kg. per hour) of preclimacteric avocado fruits stored at 68° F. after fumigation at 70° F. with 2 and 4 pounds of methyl bromide per 1000 cubic feet for 2 hours.

2-pound dosage, and only 17 for those receiving the 4-pound dosage. Surface discoloration appeared on the fruit fumigated with the 4-pound dosage.

DISCUSSION.—The respiration rates of avocado fruits held at a temperature of 68° F. after fumigation can be used to evaluate objectively the physiological response of avocado fruit varieties to various fumigation treatments. In general, the higher the respiration rate and the greater the amount of cumulative carbon dioxide produced, the faster softening occurs and the shorter the shelf life of the fruit. The respiration rate one day after fumigation can be used to predict the effect of the fumigation treatment upon the subsequent rate of softening, shelf life, and overall quality of the fruit. That is, fruits showing a respiration rate 1½ to 2 times that of comparable untreated fruits usually soften rapidly, thus shortening the shelf life, and in many cases develop objectionable surface discoloration. The occurrence of the climacteric rise and the days required to reach the peak relative to the untreated fruits are definite criteria of the physiological response of the fruit. Softening of avocado fruits has been definitely associated with the climacteric rise.

Although the cumulative carbon dioxide production is presented for only one fumigant, it was calculated for the other fumigants used. In all cases, the cumulative carbon dioxide production was a definite asset in evaluating the treatments.

SUMMARY.—The respiratory response of avocado fruits to various fumigation treatments used to sterilize fruits against eggs and larvae of fruit flies was studied. The fumigants tested were ethylene dibromide, ethylene chlorobromide, and methyl bromide. The respiration rate is discussed in terms of its value as a rapid objective criterion of the physiological and physical response of avocado fruits to fumigation. In all cases fumigation resulted in a striking stimulation in the rate of fruit respiration, and in general, the higher the rate of carbon dioxide production, the faster was the rate of deterioration. The degree of stimulation was related to the dosage of the fumigant, except for the 2- and 3-pound dosages of ethylene dibromide for 2 hours and the 1½-pound dosage for 4 hours, all of which produced essentially the same amount of stimulation.

Data presented here indicate that the initial rate of respiration of avocado fruit and the change in rate during storage is a rapidly obtained objective index to the effect of fumigation upon the subsequent response of the fruit during storage.

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Persistence of Chlorobenzilate Residues in Mature Lemon Fruits[1,2]

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As discussed by Gunther & Blinn (1955), there appear to be two principal types of residues on and in treated plant parts. When log residue or percentage of loss or retention of residue is plotted against days after application, a single straight line is obtained with most substrates. With citrus peel as the substrate, however, two intersecting straight lines are usually obtained. The single line represents the summation of degradation[3] and persistence behavior, whereas the initial straight line of an intersecting pair is considered to represent degradation, and the line of subsequent behavior to represent the persistence phenomenon. Obviously, the two types of behavior overlap with citrus peel, but it is convenient for purposes of discussion to consider that degradation curves demonstrate graphically the sum of the processes usually classified as weathering, and that falling persistence curves undoubtedly demonstrate enzymatic or other alterations of the parent molecule within the plant part (Gunther & Blinn 1955).

The general logarithmic character of the degradation and persistence behavior of organic insecticides and acaricides on and in plant parts has been amply demonstrated (Gunther *et al.* 1946, Decker *et al.* 1950, Gunther & Blinn 1955, Satyanarayana 1954). Persisting residues on and in citrus fruits that have been evaluated by means of such graphs include Aramite, Ovotran, DDT, dieldrin, EPN, parathion, and Sulphenone (Gunther & Blinn 1955).

In the broad sense degradation and persistence behavior may be expressed in terms of half life values, where the half life is the time required for half of a given quantity of material to react or dissipate. Thus, the persistence half life values in days for the above pest-control ma-

[1] Paper No. 847, University of California Citrus Experiment Station, Riverside. Accepted for publication January 3, 1955.

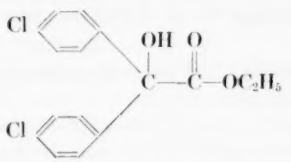
[2] The assistance of R. C. Blinn, J. O. Complin, and M. J. Jesser is gratefully acknowledged.

[3] As commonly defined to mean reduction of amount or wearing away of a substance.

terials in citrus peel are as follows: Aramite, 7–8; Ovotran, 10; DDT, 30–40; dieldrin, 8–10; EPN, *ca.* 80; parathion, 60–80; and Sulphenone, 9–12. Of these, Aramite, Ovotran and Sulphenone are generally recognized as acaricides rather than as insecticides.

Residues of the acaricide Chlorobenzilate in contrast to the residual behavior of these three acaricides, are as long lived in the field in the peel of mature lemon fruits as some of the insecticides named above, for Chlorobenzilate possesses a half life of 60–80 days. This long half life has been established by two methods of chemical assay (ultraviolet and colorimetric), as reported in this paper, and is supported by biological assay with the citrus red mite, *Metatetranychus citri* (McG.), on lemons.

In purified form the acaricide Chlorobenzilate possesses the following analytical properties:



Molecular weight: 325.2

λ max. (95 per cent ethyl alcohol): 276.2, 272, 266.5, 259.7, 254.5, 227 mμ.

λ max. (2,2,4-trimethylpentane): 275, 266.2, 259.3, 229.5 mμ.

A_s (95 per cent ethyl alcohol): 661 at 266 mμ.
17,940 at 225 mμ.

A_s (2,2,4-trimethylpentane): 741 at 266 mμ.
19,620 at 230 mμ.

Other physical properties have been listed by Blinn *et al.* (1954) and by Gunther & Blinn (1955). The compound is readily hydrolyzed to the substituted benzilic acid by strong alkali.

MATERIALS AND METHODS.—Mature lemon trees were sprayed on March 31, 1954, with either 1 or 3 pounds of a 25 per cent wettable powder formulation of Chlorobenzilate per 100 gallons of water. Applications were made as conventional sprays, using a high-pressure reciprocating pump and manually operated spray guns. Final sprays were applied at the rate of approximately 1000 gallons per acre.

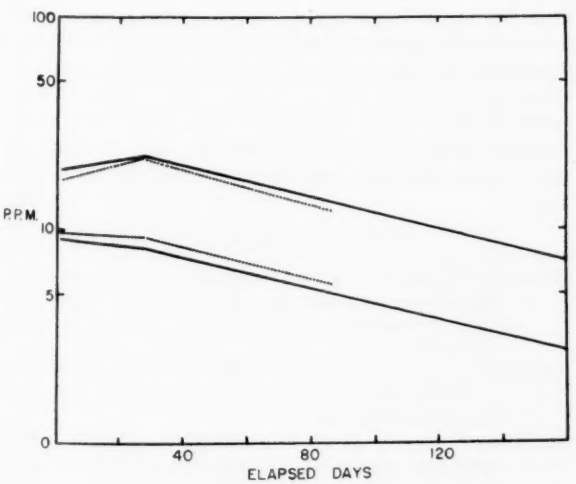
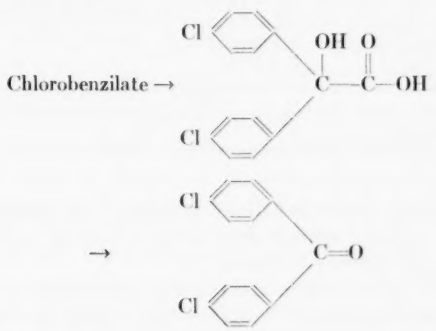


FIG. 1.—Smoothed persistence curves for Chlorobenzilate residues in peel of lemons (upper curves) after spraying with ¾-pound actual compound per 100 gallons and (lower curves) after spraying with ½-pound actual compound per 100 gallons (continuous line represents determination by ultraviolet method; dotted line, colorimetric method).

Lemon fruit samples for chemical assay of residues were collected 2, 4, 8, 15, 29, 64, 90, 120, and 141 days after treatment. Eight fruits (2 from each quadrant) were picked from each of the four trees per plot, and the resulting 32 fruits were processed as a unit. The two replicates for each treatment were processed separately. Control (untreated) samples were collected at each interval.

The fruits were washed, peeled, and processed with petroleum ether in the usual manner (Gunther & Blinn 1955) to afford final, hydrolyzed stripping solutions, which were oxidized with buffered permanganate so as to convert the acaricide present to 4,4′-dichlorobenzophenone (Blinn *et al.* 1954):



On the near-microgram scale the over-all efficiency of the above two-stage reaction is at least 87 per cent. Since the two stages also represent very effective cleanup operations, quantitative determination of the final ketone above confers a high degree of specificity to this method. For comparative purposes the final ketone was determined both by its ultraviolet absorption at 264 mμ and by a colorimetric method through its visible absorption as the 2,4-dinitrophenylhydrazone at 510 mμ (Blinn *et al.* 1954).

Lemon fruits for biological assay of residues were picked from the same trees at the same time as the fruits for chemical investigation. One fruit from each quadrant of each of the four trees per plot comprised the plot sample, making a total of 16 fruits for each of the two replicates. Twenty-five citrus red mites were exposed to the residue available on and in the peel of each field-treated fruit for 96 hours under laboratory conditions (70° F. and 50 per cent R.H.), after which the live and dead mites were counted under 20X magnification, and the net percentage mortality was ascertained.

RESULTS.—*Chemical Assay.*—Since the conversion of Chlorobenzilate to the ketone provides additional cleanup and consequent specificity, it is of interest to compare the residue data obtained by the two methods (ultraviolet and colorimetric) in parallel determinations, as collated in table 1 and plotted in the usual manner in figure 1.

RESULTS.—*Biological Assay.*—The percentage mortality of the citrus red mite upon exposure to aged field

Table 1.—Persistence of Chlorobenzilate in peel of field-sprayed, washed lemons determined by two residue methods.

Days After Application	Residue, p.p.m.[a] Ultraviolet Method	Colorimetric Method
	¼-lb. Actual/100 Gal. Applied	
Before[b]	0.0, 0.0, 0.1	0.0, 0.0, 0.0
1	11.0, 10.4	9.0, 9.7, 10.0
2	8.0, 9.0	9.8, 9.1, 9.8
14	8.3, 8.8	10.5, 9.1, 9.8
28	8.2, 8.4	8.8, 9.3, 8.8
60	6.0, 5.4	6.7, 5.8, 6.7
85	4.6, 5.0	4.9, 4.7, 4.9
113	3.3, 3.6	— — —
140	2.3, 2.8	— — —
	¾-lb. Actual/100 Gal. Applied	
Before[b]	0.0, 0.0, 0.1	0.0, 0.0, 0.0
1	16.6, 17.2	13.8, 14.8, 16.0
2	19.6, 18.4	16.6, 16.8, —
14	18.4, 17.4	18.9, 19.0, 19.0
28	20.1, 19.1	19.0, 19.4, 19.0
60	13.8, 14.0	12.4, 14.7, 14.7
85	14.3, 11.2	11.1, 12.4, 11.6
113	8.0, 9.0	— — —
140	6.5, 7.5	— — —

[a] All 32 control peel samples contained less than 0.1 p.p.m. of apparent Chlorobenzilate each.
[b] Sampled a few minutes before trees were sprayed.

residues of Chlorobenzilate on sprayed, unwashed lemon fruits, plotted against days after treatment, is shown in the usual manner in figure 2.

Discussion.—The residue data presented in table 1 and plotted in figure 1 were obtained from lemons which had been scrubbed with a detergent solution prior to being processed. This procedure was dictated by the desire to obtain residue data for fruit as prepared by the housewife. Consequently, the initial two or three determinations do not represent the true initial deposit and total residue picture and cannot be interpreted in terms of the usual degradation curve.

By analogy with the other insecticides and acaricides cited earlier, and by extrapolating the present curves, it is estimated that the initial deposit of Chlorobenzilate was about 40 p.p.m. for the ¾-pound dosage and perhaps 20 p.p.m. for the ¼-pound dosage. It can be concluded, however, that very rapid initial penetration of the Chlorobenzilate into the peel tissues occurs, and that penetration continues for at least 28 days.

That the acaricide does not penetrate through the peel and into the juice or "pulp" portions of the treated lemons in appreciable amounts was demonstrated by 36 companion analyses of the pulp portions (Gunther & Blinn 1955) of the lemon samples used for data in table 1. Fourteen and 28 days after treatment there were traces of Chlorobenzilate in the pulp, as found by both methods, amounting to less than 0.2 p.p.m. for the ¾-pound dosage and to less than 0.1 p.p.m. for the ¼-pound dosage. Subsequent samples of pulp were indistinguishable from control samples.

After 28 days the residue behavior for both dosages is the usual persistence behavior, with an indicated half life of 60–80 days, and the parallelism of the two dosage curves lends further confidence to the first-order reaction

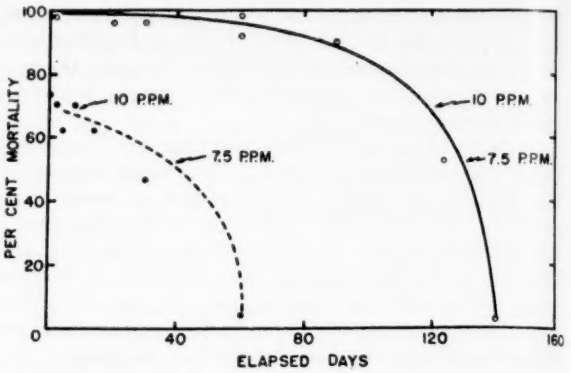


Fig. 2.—Time-mortality curves for the citrus red mite on lemons field-sprayed with Chlorobenzilate (continuous line represents the ¾-pound dosage; dotted line, ¼-pound dosage).

rate characteristics of the persistence phenomenon as applied to citrus peel.

From figure 2 it is apparent that the ¾-pound dosage was as effective after 120 days as the ¼-pound dosage was initially, the latter having an *effective* initial deposit (Gunther & Blinn 1955) of about 10 p.p.m. of Chlorobenzilate (from Fig. 1). Similarly, after 130 days the larger dosage afforded a residue of about 7.5 p.p.m., a residue which existed after 38 days with the smaller dosage.

The field performance of Chlorobenzilate as an acaricide for control of the citrus red mite has not been commensurate with its residual behavior in citrus peel alone, probably because at economical dosages such as ¼ pound/100 gallons of spray it is not highly toxic to this species of mite (see Fig. 2). Complete field performance data for Chlorobenzilate on this and other species of mites attacking citrus are being published separately. The present biological data have been included to support the chemically analyzed residue picture as well as to indicate the toxicity limitations of Chlorobenzilate to the citrus red mite.

Summary.—Two methods of chemical assay, supported by biological assay with the citrus red mite, demonstrate that the acaricide Chlorobenzilate possesses a half life of 60–80 days under field conditions on and in the peel of sprayed lemons. Chlorobenzilate does not penetrate in appreciable amounts into the juice portions of treated lemons.

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Control of Mites on Citrus with Chlorobenzilate[1]

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The citrus bud mite, *Aceria sheldoni* (Ewing), is one of the most serious pests of lemons in the coastal areas of California. In 1941, Boyce & Korsmeier found that petroleum oil sprays were effective in control of this mite pest, and since that time these sprays have been largely relied on for control. In citrus culture, however, there are many situations in which it is inadvisable to apply petroleum oil sprays. Therefore, studies directed toward finding a substitute chemical control have been in progress at this station for several years.

Jeppson (1952a) reported tests with various insecticides and acaricides and found Aramite the most feasible substitute for oil sprays, but indicated that this material was less effective in controlling citrus bud mite than the conventional oil applications.

The citrus rust mite, *Phyllocoptruta oleivora* (Ashm.), and the citrus flat mite, *Brevipalpus lewisi* (McG.), may be effectively controlled by applications of sulfur during the winter and early spring months. However, sulfur may result in phytotoxicity if applied during the summer months when the populations of these mites cause injury to the developing fruit.

In initial field screening tests Chlorobenzilate was particularly effective in control of citrus bud mite and the citrus flat mite. Because of this initial promise of effectiveness, together with the importance of the citrus bud mite as a pest of lemons in California, more of the studies made to date with this material were with this pest. However, in order to ascertain the value of Chlorobenzilate as a general acaricide for citrus mites its effectiveness was evaluated against the citrus red mite, *Metatetranychus citri* (McG.), and the Yuma mite, *Eotetranychus yumensis* (McG.). Ovotran was used as a standard of comparison in these evaluations.

Methods and Materials.—All spray applications for these studies were made in commercial citrus orchards. Unless otherwise indicated, sprays were applied by means of standard orchard spray rigs equipped with high-pressure reciprocating-type pumps. A tower was used where adequate coverage was not readily obtained by spraying from the ground. Boom spray applications were made with an oscillating boom sprayer equipped with a high-pressure centrifugal pump.

Dusts and spray-blower applications were made by means of a fishtail type, high air-volume blower equipped with spray pump capable of discharging spray into the air stream in the fishtail under 300 pounds pressure. Dusts were applied at 100 pounds per acre. The spray blower applied 300 gallons of spray per acre.

Plots treated for citrus bud mite and citrus red mite control studies consisted of eight sample trees with adequate buffer rows to allow for spray or dust drift. Each treatment was applied to two plots in each grove. Bud mite populations were evaluated by ascertaining the percentage of buds infested on the new growth that developed after treatment, as described by Jeppson (1952b). In order to ascertain the population through the period of movement of citrus bud mites to new buds, samples consisting of 15 terminals per plot were taken four times, at 2-week intervals, until the buds from 60 terminals per plot had been sampled. Five buds were examined per terminal, making a sample total of 300 buds per plot (600 per grove).

Citrus red mite populations were evaluated by counting the adult mites on 32 leaves on each of 8 trees in each plot, as described by Jeppson (1951). Results are reported as average number of mites per tree (32-leaf sample).

To evaluate populations of the citrus flat mite, four areas of the size visible through a 10× hand lens were examined per fruit. Eight fruits per tree were examined from eight trees per plot. Results are reported as percentages of hand-lens fields infested with mites.

Populations of Yuma mite were ascertained by counting the mites along the entire length of the midrib on both the upper and the lower surface of the leaf by means of a wide-field binocular microscope equipped with 20X magnification. All mites passing under the range of view of the microscope as the midribs were examined were included in the sample count. Results are reported as percentages of leaves infested.

The citrus rust mite population was estimated by examining 50 fruits per plot by means of a 10× hand lens.

Discussion of Results.—Results of field experiments in 10 lemon groves in which treatments with Chlorobenzilate formulations and with standard 1.75 per cent petroleum oil were evaluated for effectiveness in control of citrus bud mite are reported in table 1. In these experiments dosages of 4 to 8 ounces of actual Chlorobenzilate per 100 gallons of spray generally resulted in more effective control of citrus bud mite than a spray of 1.75 gallons of a light-medium emulsive petroleum oil (California classification), which has been the standard treatment for controlling this pest in California. When applied at 4 or 8 ounces per 100 gallons of spray, there was little or no difference between the control obtained with the wettable powder formulation of Chlorobenzilate and that obtained with the emulsifiable concentrate. The difference between the average infestations at the 4- and 8-ounce dosages of each formulation was not over 3.5 per cent, which does not appear to justify use of the higher dosage (Table 1).

In four field experiments in which the wettable powder formulation was applied at dosages of 2, 4, and 8 ounces, 2 ounces per 100 gallons resulted in as effective control of the citrus bud mite as 4 ounces, and in two of the four trials 2 ounces were as effective as the 8 ounces (Table 2). In other tests not reported in the tables, dosages of 16 ounces per 100 gallons were no more effective than the lower dosages.

Since the citrus bud mite lives in the buds under the bud scales or under fruit buttons, populations are often not effectively reduced by spray applications. The use of additives to increase wetting or penetration into the protected places appeared to be a possible approach to increasing the effectiveness of the treatment. However,

[1] Paper No. 848, University of California Citrus Experiment Station, Riverside. Accepted for publication January 3, 1955.

Table 1.—Relative effectiveness of spray formulations of Chlorobenzilate and of conventional petroleum oil treatments against the citrus bud mite in 10 southern California lemon groves.

Formulation	Dosage per 100 Gals.	Per Cent Buds Infested[a] in Groves										
		1	2	3	4	5	6	7	8	9	10	Mean
Chlorobenzilate (actual) Wettable Powder	4 oz.	16.5	12.0	8.0	27.5	12.5	18.5	46.0	30.0	9.5	15.0	19.5
	8 oz.	16.0	8.0	5.0	11.0	7.5	13.8	40.0	34.5	9.0	22.5	16.7
Emulsifiable concentrate	4 oz.	17.5	11.5	12.0	26.0	12.5	16.8	36.0	21.0	14.0	19.5	18.7
	8 oz.	16.5	18.0	13.0	14.0	7.5	17.5	21.5	11.5	13.0	19.5	15.2
Petroleum oil (light-medium emulsive)[b]	1.75 gal.	34.3	24.0	5.8	47.5	27.5	43.5	36.0	30.0	45.5	23.5	31.8

[a] Averages of two plots (600 buds per grove).
[b] California classification.

Table 2.—Influence of dosage of Chlorobenzilate wettable powder formulation on control of citrus bud mite.

Ounces Actual Chlorobenzilate per 100 Gallons	Per Cent Buds Infested[a] in Groves				
	A	B	C	D	Mean
2	12	14	23	21	17.5
4	23	16	27	20	21.5
8	25	22	8	10	16.3

[a] Averages of two plots (600 buds per grove).

the addition of polyglycol[2] to the wettable powder formulation as an additional wetting and spreading agent did not increase the effectiveness of the formulation in controlling the citrus bud mite (Table 3). Nor was control improved when petroleum oil or kerosene was used as a supplement to the emulsifiable concentrate formulation of Chlorobenzilate for the purpose of increasing penetration under bud scales (Table 4).

At equivalent amounts of actual toxicant per unit (per 100 gallons or per acre) of application, Chlorobenzilate was less effective than Ovotran or the standard petroleum oil applications in the control of citrus red mite (Table 5). Gunther *et al.* (1955) have shown that Chlorobenzilate spray residues persist for an unusually long time on fruit surfaces, but that relatively high surface resides are required to kill citrus red mite. It would appear that the relative ineffectiveness of Chlorobenzilate for control of the citrus red mite is due to its lower toxicity to this mite species.

[2] Thiosolve 42–1A.

Table 3.—Influence of additional spreading agent on control of citrus bud mite by wettable powder formulation of Chlorobenzilate.

Formulation	Dosage per 100 Gal.	Per Cent Buds Infested[a] in Grove							
		A	B	C	D	E	F	R	Mean
Chlorobenzilate WP	4 oz.	12	23	27	29	5	19	13	18.3
Chlorobenzilate WP + polyglycol[b]	4 oz. 2 oz.	23	19	20	32	4	14	12	17.7

[a] Averages of two plots (600 buds per grove).
[b] Thiosolve 42–1A.

Table 4.—Influence of the addition of petroleum oil or kerosene to emulsifiable formulations of Chlorobenzilate on control of citrus bud mite.

Formulation	Amount Actual per 100 Gallons	Per Cent Buds Infested[a] in Grove				
		A	B	C	D	Mean
Chlorobenzilate EC[b]	4 oz.	18	29	16	12	18.8
Chlorobenzilate EC + Petroleum oil (light-medium emulsive)	4 oz. 1.75 gal.	16	17	18	—	17.0
Chlorobenzilate EC + Kerosene	4 oz. 1.75 gal.	19	28	18	14	19.8
Petroleum oil (light-medium emulsive)	1.75 gal.	30	27	23	26	26.5

[a] Averages of two plots (600 buds per grove.)
[b] Emulsifiable concentrate formulation.

Table 5.—Relative effectiveness of Chlorobenzilate, Ovotran, and petroleum oil against the citrus red mite on citrus trees.

Grove	Formulation[a]	Dosage per 100 Gals. or per Acre[b]	Av. No. of Mites per 32-Leaf Sample (Days After Treatment)		
			76	101	177
	Conventional Spray Applications				
A	Chlorobenzilate WP	4 oz.	21	32	37
	Chlorobenzilate WP	8 oz.	2	50	31
	Chlorobenzilate WP	12 oz.	2	2	46
	Chlorobenzilate EC	4 oz.	13	112	—[c]
	Chlorobenzilate EC	6 oz.	2	11	50
	Chlorobenzilate EC	8 oz.	1	13	30
	Ovotran WP	6 oz.	1	4	28
			23	72	
B	Chlorobenzilate EC	4 oz.	2.0	23.0	
	Ovotran WP	4 oz.	0.4	0.4	
			32	77	
C	Chlorobenzilate WP	4 oz.	22.7	17.3	
	Petroleum oil (L-M E)	1.75 gals.	0.7	5.0	
	Spray-Blower Applications				
			76	101	
A	Chlorobenzilate WP	4 lb.	0	26.0	
	Chlorobenzilate EC	1 lb.	5.0	54.0	
	Ovotran WP	4 lb.	0	2.0	
			23	72	
B	Chlorobenzilate EC	4 lb.	2.0	23.0	
	Ovotran WP	4 lb.	0.4	0.4	

[a] WP = wettable powder; EC = emulsifiable concentrate; L-M E = light-medium emulsive (California classification).
[b] Equivalent amounts actual toxicant per unit (per gallon or per acre).
[c] Re-treated prior to this sampling.

Table 6.—Relative effectiveness of Chlorobenzilate, Ovotran, and Aramite against *Eotetranychus yumensis* (McG.) on lemon trees.

Formulation	Actual Toxicant (Oz./100 Gal.)[a]	Per Cent Leaves Infested 6/16	7/24	11/11
Chlorobenzilate 25%, WP	4.0	16	4	38
	8.0	14	7	24
Aramite 15%, WP	2.4	35	4	31
	4.8	13	4	18
Ovotran 50%, WP	4.0	1	8	15
	8.0	3	5	26

[a] Applied April 16, 1953.

In initial field evaluations Chlorobenzilate approached the effectiveness of Ovotran and Aramite for control of the Yuma mite (Table 6). Further study will be necessary, however, to judge adequately the value of either of these acaricides for control of this mite pest. A 4 per cent Chlorobenzilate dust applied at 100 pounds per acre was relatively ineffective, as compared with sulfur dust applied at 100 pounds per acre, in control of the citrus rust mite (Table 7). However, when Chlorobenzilate was applied as a spray at 4 pounds per acre by the use of air-blast equipment employing 300 gallons of spray per acre, the resulting control was as satisfactory as similar sulfur spray applications using 30 pounds per acre, or as sulfur dusts at 100 pounds per acre (Table 7).

Chlorobenzilate was more effective than DMC, Ovotran, Sulphenone, or Aramite in reducing high populations of the citrus flat mite (Table 8). At 2.5 pounds per acre Chlorobenzilate applications made at the early date of May 5 by spray-blower equipment resulted in less effective control of the citrus flat mite than sulfur applications at 15 or 30 pounds per acre (Table 8).

SUMMARY.—Chlorobenzilate was generally more effective than petroleum oil sprays in control of the citrus bud mite, *Aceria sheldoni* (Ewing). Summer applications of Chlorobenzilate resulted in effective seasonal control of the citrus flat mite, *Brevipalpus lewisi* (McG.), and this material also indicated promise in the control of the citrus rust mite, *Phyllocoptruta oleivora* (Ashm.), and the Yuma mite, *Eotetranychus yumensis* (McG.). Chlorobenzilate was less effective than Ovotran or petroleum oil sprays for control of the citrus red mite, *Metatetranychus citri* (McG.).

Table 7.—Relative effectiveness of various acaricides against the citrus rust mite and the citrus red mite on orange trees.

Formulation	Pounds Actual Toxicant per Acre	Method of Application 4/17/53	Population Count 9/4/53 Citrus Rust Mite	Citrus Red Mite
Sulfur dust	100	Duster	Light	Moderate
Sulfur WP+ Ovotran 50%, WP	30 4	Spray blower	Light to medium	Light
Chlorobenzilate 4% dust	4	Duster	Heavy	Moderate
25%, WP	4	Spray blower	Trace	Moderate

Table 8.—Relative effectiveness of several acaricides against *Brevipalpus lewisi* on orange trees.

Formulation	Amount Actual Toxicant	Per Cent Infestation (Hand-Lens Fields)
Conventional Spray Application (July 24, 1952)[a]	(Ounces/100 gal.)	
Chlorobenzilate, EC	8.0	0.3
Chlorobenzilate, WP	8.0	0.9
DMC	8.0	4.1
Sulphenone, WP	9.6	31.3
Sulfur, WP	16.0	10.0
Ovotran, WP	8.0	12.5
Aramite, WP	4.8	12.5
Spray-Blower Application (August 22, 1952)[b]	(Pounds/acre)	
Sulphenone, WP	6.5	Moderate
Chlorobenzilate, EC	2.0	None
Chlorobenzilate, WP	2.5	None
DMC, EC	2.0	Trace
Boom-Spray Application (May 5, 1953)[c]	(Pounds/acre)	
Sulfur, WP	15.0	None
Sulfur, WP	30.0	None
Chlorobenzilate, WP	2.5	Moderate
Chlorobenzilate, EC	2.5	Moderate
Untreated	—	Heavy

[a] Population sampled August 14, 1952.
[b] Population estimated Sept. 9, 1952.
[c] Population estimated July 29, 1953.

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A Pilot Study of Area Larval Control of Black Flies in Guatemala[1]

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Black flies (Diptera: Simuliidae) are considered to be the vectors of human onchocerciasis, which in the Western Hemisphere is endemic in parts of Guatemala, Mexico, and Venezuela. At a laboratory in San Pedro Yepocapa, Guatemala (Fig. 1), medical, parasitological, and entomological investigations have been carried out on various aspects of this disease. This work was made possible through the cooperation of the Laboratory of Tropical Diseases, National Institutes of Health and the Pan American Sanitary Bureau.

The principal anthropophilic species in the disease zone are: *Simulium ochraceum* Walker, *S. metallicum* Ballardi, and *S. callidum* (Dyar and Shannon). In 1951, investigations were undertaken to find a means of controlling these vectors through the use of insecticides. The first phase of this study was the establishment of a laboratory larvicide screening program, whereby a large number of chemicals of unknown toxic properties, as well as various insecticides, could be evaluated prior to use in the field. These results and the technique employed have been reported by Lea & Dalmat (1954). The second part of the work involved experiments to determine the effectiveness of several promising materials against black fly larvae under actual field conditions (Lea & Dalmat 1955). The final phase of these investigations was the small-scale experiment in area control, herein reported. The area chosen was the Municipality of Yepocapa in which the town and the laboratory were located.

In Kenya Colony, Africa, Garnham & McMahon (1947) had been successful in such a project by controlling

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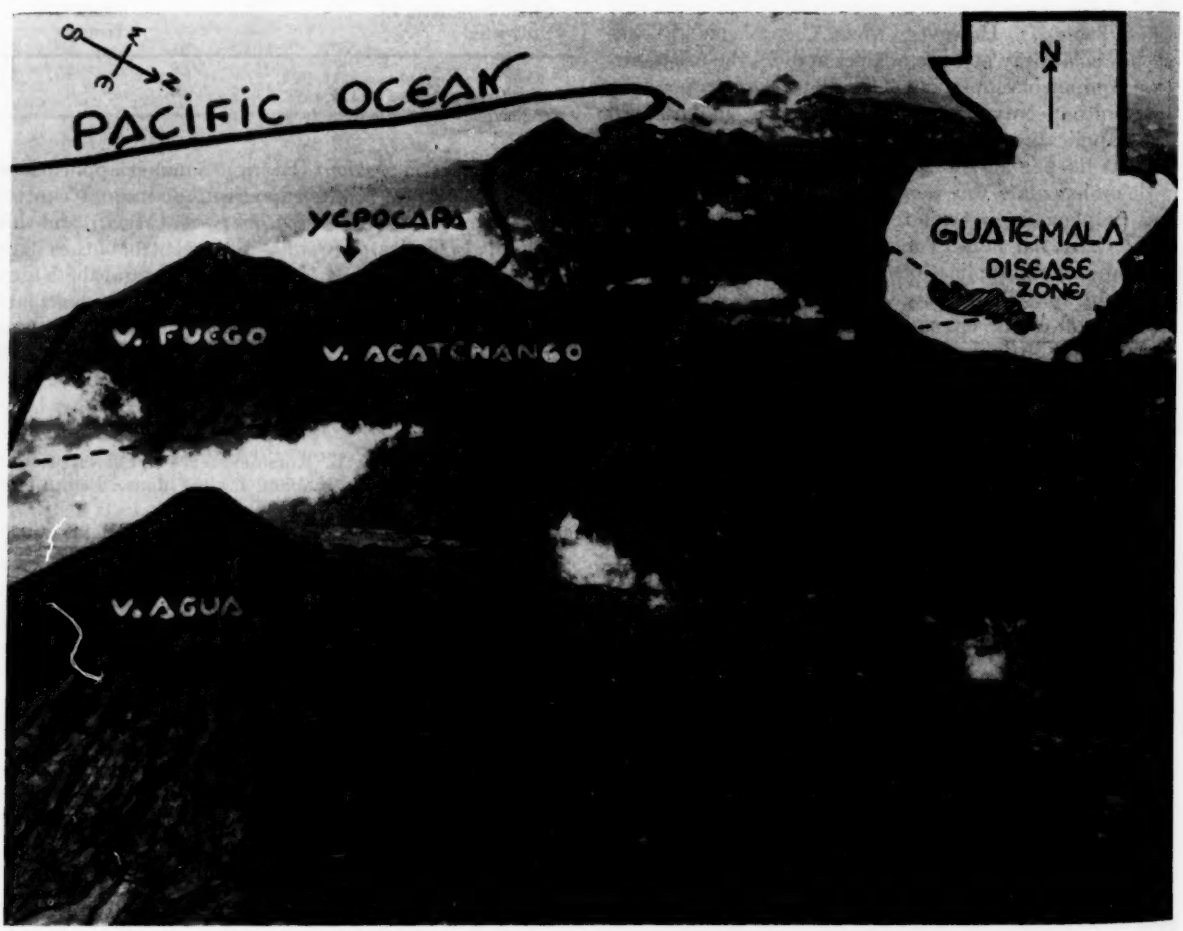

FIG. 1.—Aerial photograph of Pacific Cordillera in Guatemala. That part of the endemic zone of onchocerciasis which is visible has been outlined, and its orientation indicated by the map insert. The town of San Pedro Yepocapa in which the laboratory was located is on the opposite side of the volcano Acatenango.

the larvae of *Simulium neavei*, vector of onchocerciasis in that region, with repeated treatments of DDT at 2 p.p.m. for 3 minutes in sections of two rivers, totaling 15 miles in length, where breeding occurred.

A similar area-wide campaign against black flies had been undertaken by Hocking & Richards (1952) who surveyed 163 streams and rivers in a 200 square mile circular area around the Goose Bay, Labrador airport, and found 77 streams which required larvicidal treatment. Applications of DDT in fuel oil were made at the rate of 0.1 p.p.m. minutes[3] from a helicopter or the ground. However, infiltration of adults from outside the area was troublesome, although a worthwhile reduction in number was obtained 4 to 6 miles from the center.

An adequate description of terrain, climate, and methods of calculating current flow in rivulets and streams has been presented in the preceding paper of this series (Lea & Dalmat 1955).

STREAM LARVICIDING.—The area-wide larviciding of breeding streams in the Municipality of Yepocapa was carried out between December 1, 1952 and May 15, 1953. For reasons to be explained later, the actual treatment of streams was carried out only for the first 3 months of this period, while biting rate records of the adult black flies were continued into May.

The program was primarily a pilot study to determine the problems which might be encountered in a future large scale attempt to control the vectors. It was realized that the time available for this study would not permit the work to be carried on long enough or to be extended over a sufficiently large area, that any significant reduction of the adult population could be effected. However, even during the short period the project was operating, sufficient information was obtained that methods and plans could be suggested for a future control campaign.

The data of earlier experiments in individual streams (Lea & Dalmat 1955) had established that the distance to which larvae were cleared from a stream varied. For a particular concentration and application period of DDT, depending on the stream-volume at the point of treatment, the greater the volume the greater the distance of kill. Thus, in the smallest rivulets DDT at the rate of 0.1 p.p.m. for 3 minutes was effective for 100 to 200 feet, whereas in a stream of 5000 gallons per minute the effect carried for about 2 miles downstream. However, DDT at this concentration did reach a limit at about 5000 gallons per minute beyond which the distance of kill did not increase proportionately in any of the larger streams treated. The distance of kill for DDT at 2 p.p.m. for 3 minutes and 0.1 p.p.m. for 60 minutes in streams ranging from 10 to 100,000 gallons per minute had also been determined. No limit to the distance of kill in the largest streams treated was encountered.

As the rivulets were generally not over 200 feet in length, one application at their source was usually sufficient to rid them of larvae. The larger streams were treated at several points along their course with 0.1 p.p.m. DDT for 3 minutes, the distance between treatments depending on the distance of kill attainable with this concentration and on the particular stream-volume as calculated at previous point of treatment. Since each main stream was followed downstream in order to treat all its tributaries, even those with volumes greater than 5000 gallons per minute could advantageously be given repeated treatments of 0.1 p.p.m. DDT for 3 minutes, although each treatment would not be effective for more than 2 miles from the point of treatment. However, in the longer streams and in places where inaccessibility hindered more frequent applications, either 0.1 p.p.m. DDT for 60 minutes or 2 p.p.m. for 3 minutes were used effectively since the distance of kill was greater.

The field personnel were always sent out in pairs, each pair assigned to treat the tributaries of one or two of the major stream systems. The work was begun at the headwaters of each main stream and each of its tributaries having larvae was followed to its source and treated. If the tributaries were branched, as many of them were, each branch had to be treated. In a long rivulet which required interval treatments and which had branches joining it, the volume at each point of treatment could satisfactorily be estimated by taking the sum of the volumes of all the upstream branches which entered it.

Each pair marked the number and volume of each tributary on a convenient rock or log near its entrance to the main stream, at the same time indicating these on a rough schematic map. A calibrated metal canal (Lea & Dalmat 1955) was used to calculate the volumes. For the sake of ease in dispensing the DDT emulsion, it was prepared in 4 cc. vials with either black or red screw tops. Knowing that a half-full black-topped vial supplied sufficient concentrate to treat a rivulet with a volume of 10 gallons per minute, and that the contents of a half-full red-topped vial was adequate for a 50 gallons-per-minute stream, the operators could treat any of the small breeding streams in the area by applying the contents of an appropriate combination of one-half, three-fourth, or completely full vials (Fig. 2a). The necessary volume of DDT concentrate was emptied into a tin can and then diluted with stream water to a known level that permitted the resulting emulsion to be dispensed through a rubber-tube siphon in 3 minutes.

Since it was necessary to mark tributaries and calculate their volumes only at the time of the first application, subsequent treatments could be made in a much shorter period of time. Thus, it was possible to increase the number of streams treated by each pair, thereby gradually expanding the total area under control. Because of the continuous breeding of the black flies throughout the year, their relatively short life cycle and their extensive flight range, 3 weeks after the first application streams would be reinfested and require retreatment. In all, some 1500 rivulets and streams were under continuous treatment in an area about 80 square miles covering nearly all the Municipality of Yepocapa.

During the early weeks of this program, one group was sent to the field to check on results of previous treatments and to retreat streams in which larvae were found. Later, when it was determined that the first treatments were effective in eliminating the larvae from the streams, only occasional spot-checks were made. The maps made by the field personnel were returned to the laboratory and the data recorded. From the laboratory's master map, a list was prepared for each of the main stream systems giving only the number of each tributary and its volume. The

[3] For explanation, see Hocking *et al.* (1949).

FIG. 2.—(a) Larvicide treatment with DDT emulsion, at the rate of 0.1 p.p.m. for 3 minutes, is being siphoned from a tin can into a stream. Vials of emulsion concentrate to be used in stream treatment are carried in a canvas belt with pouches. (b) Adult population fluctuations recorded by means of daily collections of biting flies, in test tubes from subject, seated.

number of the stream on the list was underlined in color to indicate that it entered the main stream from the right, the numbers not underlined were streams entering from the left, going downstream. This aided in orientation in the field, so that streams would not be overlooked. Generally, one member of the pair treated only the tributaries entering from one side.

At the same time the larviciding was begun, four adult collecting stations were set up and maintained to sample the adult biting population. Later, as personnel became available, four more stations were added. Each of these required a subject, stripped to the waist and with legs exposed, and a second individual who collected in test tubes flies biting the subject (Fig. 2b). The flies were most

Table 1.—Adult black fly population during area-larvicide experiment as determined by biting rate collections made at eight stations.[a]

DATE	AVERAGE NUMBER OF FLIES COLLECTED AT							
	Panajabal[b]	Rosario[b]	Queleya	Conchita	Recreo	Cuchilla	Nimaya	Montevideo
Nov. 30[c]–Dec. 27	—	—	128.6	231.4	344.6	63.0	240.7	272.2
Dec. 28–Jan. 24	—	—	114.5	304.3	228.8	68.0	168.3	204.3
Jan. 25–Feb. 21[d]	198.0	275.0	88.2	146.0	115.0	12.0	80.5	91.5
Feb. 22–Mar. 21[e]	174.4	254.5	110.3	187.2	144.0	23.4	165.5	103.6
Mar. 22–Apr. 18	117.6	150.4	81.4	119.2	91.8	13.0	158.7	78.2
Apr. 19–May 9	74.0	66.0	21.5	60.0	55.7	10.7	102.7	38.0

[a] Flies collected from subject between 8 A.M. and 11 A.M., collections scheduled twice per week at each station and reported as average number of flies collected during each 4-week period from December to May.
[b] Control stations considered outside the treated area so that population is unaffected by larviciding.
[c] Larviciding begun.
[d] Larviciding ended.
[e] Note population increases after end of larviciding only at stations within treated area.

active during the early morning so that collections were made from 8 A.M. to 11 A.M., at least twice a week at each station.

RESULTS AND DISCUSSION.—The fluctuations in the biting population between December and May are shown in table 1. The actual larviciding was continued from December to the first of March, and coincident with the cessation of insecticide applications, there was a rise in the adult population. This increase was followed by a gradual decline until the time the last records were taken in May.

There were many factors, aside from the physical difficulties, which tended to complicate any attempt to control black flies over a relatively small area. Not the least of these is the infiltration of flies from areas outside the treatment zone. Dalmat (1952) determined that flies would travel for distances up to at least 10 miles from their release point. Winds also were undoubtedly an important factor in migration and certainly account for much of the daily fluctuations in adult biting rates experienced in Guatemala.

It was unfortunate that the work could not have been initiated in time to prevent the build-up in population which occurs in November and December, just after the termination of the rainy season. The first treatments were made on December 1, 1952, the biting rate counts having been started the day before.

In a previous study (Dalmat 1955) made at this laboratory during a year when no insecticides were being used, it was found that the biting rates declined gradually over the months of December to May. If, as has been mentioned, there is a relationship between stream size and the size of the larval population it can support, it seemed reasonable to assume that this decline in population was the result of the concurrent drop in volume of the breeding streams in the area. Therefore, if the population declined during the period of larviciding, it would be, at least in part, due to the normal population decline for the period, and also partly the result of the insecticide. In the short time remaining before the closing of the laboratory, it appeared that the only way to demonstrate any effect which the insecticides alone might have, was to carry on the treatments for a period of 3 months, and then to observe any changes in the adult fly population coincident with the discontinuance of larviciding. This was done, and with the termination of the treatments, it was found that the adult population which had been declining showed a rise and then began to decline again (Table 1). This was interpreted to mean that the larviciding had depressed the adult population below its normal decline; that when the applications were stopped, a build-up occurred in the "depressed population" approaching the density which the streams would normally produce at that time. In the ensuing weeks the number of favorable breeding habitats continued to fall off as the dry season progressed, resulting in the normal decline in population.

Not all the collecting stations could be set up at the beginning of the experiment, so that the data obtained at two of them, Panajabal and Rosario, do not cover the entire period. However, these stations were considered well outside the treated area and clearly show that the decline observed at them was continuous, since there was no buildup subsequent to the termination of the larvicidal treatments.

Because the natural infection rate of biting flies is low (less than 1%), there is adequate reason to believe that larvicidal control can be an effective weapon against onchocerciasis. This report deals with an experiment in only a small area for a very short period of time. A well organized program covering a large part of the disease zone and operating for several years, could undoubtedly effect a significant reduction in the adult black fly population throughout the region. The entire endemic zone in Guatemala is estimated at approximately 600 square miles.

RECOMMENDATIONS FOR A FUTURE LARGE-SCALE VECTOR CONTROL PROGRAM IN GUATEMALA.—An approach to the problem of control should include an evaluation, first, of the merits of adult and of larval control, and second, of the most economical method, either ground or aerial, of carrying out the attack on the particular stage to be controlled. Although adult black flies are highly susceptible to several insecticides, attempts in Guatemala at adult control from the ground have failed, even in relatively open and accessible coffee plantations, because of the difficulties in transportation of even a small concentrate-type sprayer[4] over the rugged terrain (unpublished data). In more densely grown areas, ground spraying would be impossible. Aerial spray equipment was not available, but insecticide applications would have to penetrate several layers of dense foliage to be effective. This would also be a problem in larval control from the

[4] Microsol #304 loaned through the courtesy of the Silver Creek Precision Corp.

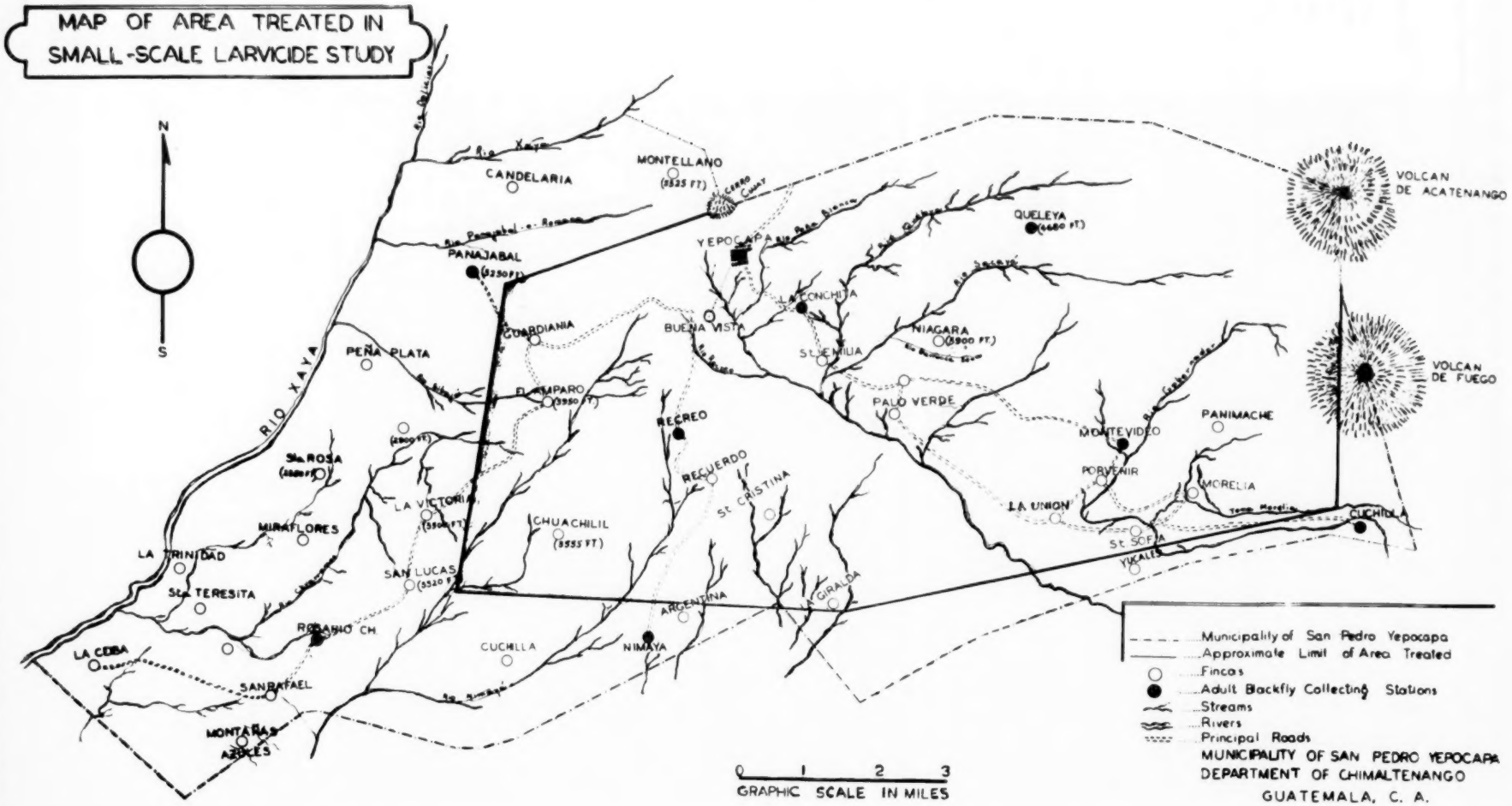

FIG. 3.—Map showing the main streams and rivers in the Municipality of San Pedro Yepocapa, Guatemala. Within the area marked, these streams and all their small tributaries were treated with DDT, to eliminate black fly larvae. Indicated are the eight adult black fly collecting stations where population samples were taken.

air, as would be the hazards for a plane following streams along the rugged slopes of the volcanoes. On the other hand, larval control from the ground, although time-consuming, is both possible and practical.

When plans for adult black fly control by ground larviciding were being made, a major obstacle in organizing the work was the lack of accurate maps of the rivers of the region. Aerial photographs of a large part of the disease zone had been taken, but not until the control program had been completed were they available for the region around Yepocapa. The accompanying map (Fig. 3) is, at best, only an approximation of the drainage systems in the area. The main streams of the area were relatively well known by the field personnel and were taken as the basis for making a systematic coverage of breeding streams. The small tributaries could only be found by following the main stream and by carefully searching for them.

It is estimated that the cost of local labor and insecticide to carry out such a program for 1 month would be between $5.00 and $8.00 per square mile. The cost of the labor required is the largest item but could be reduced if the cooperation of the plantation owners were obtained. They could furnish a certain amount of the field personnel needed for making the insecticide applications, as well as individuals to serve as subjects for the adult biting rate collections. Thus, only a nucleus of trained personnel would have to be employed directly. Although it is difficult to estimate the amount of insecticide needed, 10 to 20 gallons of 25% DDT emulsion concentrate should be adequate for 1 month in an area of 100 square miles, taking the stream volumes in the Municipality of Yepocapa as an average for the disease zone. The estimate of costs is broken down as follows:

NUMBER OF PERSONNEL NEEDED TO TREAT 100 SQ. MILES FOR 1 MONTH OF 200 WORKING HOURS	
2 Driver-Field Worker	$120
9 Field Worker	$360
1 Administrator for Central Station	$150
1 Driver-Collector	$ 50
1 Collector	$ 30
2 Subjects	$ 50
Insecticide	$ 40
Total 16	$800

NUMBER OF PAID PERSONNEL NEEDED WHEN PART OF THE LABOR IS FURNISHED BY PLANTATION OWNERS IN THE AREA	
2 Driver-Field Worker	$120
3 Field Worker	$120
1 Administrator	$150
1 Driver-Collector	$ 50
Insecticide	$ 40
7	$480

SUMMARY.—A small-scale area larviciding program was conducted in a region of Guatemala, known to be endemic to human onchocerciasis, to determine the feasibility of control of the adult black flies that serve as vectors of the disease. In the area of about 80 square miles which was included in the program, 1500 breeding streams were surveyed, treated, and retreated at intervals over a 3-month period.

The larvicide program was begun by following each of the main streams in the area downstream from its source, and systematically searching for all tributaries, determining their volume and treating them. Records of stream volumes were kept, so that by the time the streams were reinfested (21 days) retreatment was simplified.

The volume of 85% of the breeding streams was less than 100 gallons per minute. Such small rivulets were generally short enought so that one treatment at their source with DDT at 0.1 p.p.m. for 3 minutes sufficed to rid them of larvae. Long rivulets and streams up to 5000 gallons per minute and larger were given the same treatment at intervals along the stream's course. Streams with a flow greater than 5000 gallons per minute, which were inaccessible to the more frequent treatments at intervals, were given either DDT at 2 p.p.m. for 3 minutes or 0.1 p.p.m. for 60 minutes, the longer treatment being effective for a greater distance.

During the same period, and for 3 additional months, eight collecting stations were maintained to sample the adult black fly population and to note fluctuations due to the larvicidal treatments. Although the larviciding was carried on for a relatively short period, it was demonstrated that the adult biting population was depressed during that time.

On the basis of this pilot study, recommendations and estimated costs were given for a vector control campaign in a 100-mile area. The total endemic onchocerciasis region in Guatemala is considered to be 600 square miles.

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Granulated Insecticides for Control of the European Chafer in Meadows[1]

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During 1953, work was initiated with a number of granulated insecticides for the control of the European chafer, *Amphimallon majalis* (Raz.) in a meadow planted to a mixture of timothy, orchard grass, red clover and alfalfa. Since some difficulties were encountered in applying the materials and because of the interest of research workers in granulated insecticides, a preliminary report on the working properties and effectiveness of these formulations was made in an earlier paper (Gyrisco & Evans 1954). Since that time additional work has been conducted concerning the residues from granulated applications, the effects of grub control on hay yields, and the residual effectiveness of the treatments. Work was also initiated on various methods of application using hand-operated equipment, usually found on most farms, to apply granulated dieldrin as this was the most effective toxicant found in 1953.

METHODS AND MATERIALS.—The methods of preparation, the formulations, and the application of the insecticides in the experiment started in 1953 were described in the previous paper. Granulated formulations of aldrin, dieldrin, heptachlor, chlordane and toxaphene were used at the rates indicated in table 1. Five days after treatment four 1-square-foot samples of foliage were cut at cutter-bar height from each of the plots treated with aldrin and dieldrin and were air-dried, composited and subsampled. In addition to these, three 1-square-foot samples were cut from each of the plots treated with dieldrin at different heights of the foliage. These heights were designated: *top*, *middle*, and *bottom*, each sample being 3 to 4 inches in length. After curing, the appropriate samples were composited and subsampled for residue analysis. The data on the residues remaining on the foliage of the plots treated with aldrin and dieldrin are shown in table 2.

On June 3, 1954, which was approximately the optimum time for the first cutting of timothy hay for the region, the yields of all of the plots were obtained by cutting the total hay growth in each plot and weighing the foliage of each within 5 minutes of cutting. These data, expressed in pounds of hay per acre on a green weight basis are given in table 1.

On September 29, 1954 the plots were sampled for grubs by digging four 1-square-foot holes in each plot to a depth of about 9 inches and hand sorting the soil for larvae. These data are shown in table 3.

To investigate the possibility of using hand-operated fertilizer spreaders, cyclone and wheelbarrow seeders for the application of granulated dieldrin, 1/50-acre plots, replicated four times in complete randomized blocks, were laid out in a meadow infested with grubs of the European chafer. These hand methods of application of a granulated insecticide were compared with a commonly used standard method consisting of an application of dieldrin as an emulsion from a low-volume, low-pressure weed sprayer. A 2% formulation of dieldrin impregnated on Attapulgus AA, 30–60 mesh clay granules, was used at the rate of 50 and 100 pounds per acre in the plots treated with the cyclone seeder. To reduce the hazards to the operator a sheet-metal apron about 18 inches square was fitted on top of the seeder to prevent the granules from being thrown up into the face of the operator. A conventional wheelbarrow seeder with a 20-foot hopper was used to apply the granules. In addition to these treatments, dieldrin granules were mixed with enough pyrophyllite to make 8 pounds of total mix per plot and were applied with a hand-operated fertilizer spreader at that rate to give 2 pounds per acre of active

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Table 1.—Increased hay yield obtained by control of European chafer with granulated insecticides. Newark, New York. June 4, 1954.

Material	Pounds Toxicant per Acre	Average No. Larvae per[a] Sq. Ft.	Average Yield[b]
Dieldrin	2	1.0	10,965
Dieldrin	1	1.4	9,827
Aldrin	2	2.0	9,810
Heptachlor	2	2.9	9,827
Aldrin	1	3.3	6,882
Chlordane	10	3.4	8,397
Heptachlor	1	4.4	7,165
Toxaphene	20	11.2	5,332
Toxaphene	10	12.5	2,812
Untreated		23.5	860
L.S.D. at 1% level		3.14	172

[a] Coefficient of correlation (r) between average number larvae per square foot and yields of hay = −0.93, t = 7.10 indicating r is highly significant at odds greater than 99:1.
[b] Pounds per acre (green weight).

Table 2.—Residues from dieldrin and aldrin granules remaining on foliage 5 days after treatment. Newark, New York. August 11, 1953.

Material	Pounds Toxicant per Acre	Portion of Sample	Av. Residue[a] (Dry Wt. p.p.m.)
Aldrin	1	total	0.19
Aldrin	2	total	0.46
Dieldrin	1	total	0.14
Dieldrin	2	total	0.16
Untreated			0.02[b]
Untreated			0.10[c]
Dieldrin	1	top	0.1
Dieldrin	1	middle	0.32
Dieldrin	1	bottom	0.24
Dieldrin	2	top	0.20
Dieldrin	2	middle	0.18
Dieldrin	2	bottom	0.32

[a] The authors thank the Julius Hyman Division of Shell Chemical Corporation, Denver, Colorado for analyzing the samples. The phenyl azide photometric method was used for aldrin residue analysis and the reduction phenyl azide photometric method was used for the analysis of dieldrin residues.
[b] Apparent aldrin.
[c] Apparent dieldrin.

Table 3.—The residual effectiveness of granulated formulations of insecticides on the European chafer.[a] Newark, New York. 1953–54.

Material	Pounds Toxicant per Acre	Average No. Larvae/Sq. Ft.	
		1953	1954[b]
Dieldrin	2	1.0	0.70
Dieldrin	1	1.4	0.70
Aldrin	2	2.0	0.74
Heptachlor	2	2.9	0.90
Aldrin	1	3.3	1.28
Chlordane	10	3.4	0.96
Heptachlor	1	4.4	1.35
Toxaphene	20	11.2	1.38
Toxaphene	10	12.5	1.60
Untreated		23.5	2.25
L.S.D. at 1% level		3.14	0.87

[a] Applied August 6, 1953. Samples taken on October 16, 1953 and October 2, 1954.

[b] Transformed data; $x' = \sqrt{x+0.5}$ where x = average number of larvae per square foot unit.

ingredients. On the remaining plots, dieldrin emulsion was applied at the same dosage of toxicant per acre with a conventional weed sprayer using 20 gallons of spray per acre at 40 pounds of pressure per square inch. On October 2, 1954, all of the plots were sampled for grubs, in a manner already described. These data are presented in table 4.

DISCUSSION.—It is evident that summer applications of aldrin, dieldrin, heptachlor or chlordane, in granulated form and at the dosages used in this experiment, gave excellent control of two successive generations of European chafer larvae. Toxaphene, at rates of 10 to 20 pounds of actual toxicant per acre, did not give satisfactory control in 1953 and did not significantly reduce the population in 1954. Burrage & Gyrisco (1952, 1953) found that aldrin, dieldrin or chlordane, in dust or spray form, gave satisfactory control of European chafer larvae at equivalent dosages, so it is apparent that granulated formulations of these insecticides are equally effective in controlling, at least, two generations of the larvae.

Probably a better criterion of the effectiveness of the materials is the data on yields. As may be seen in table 3 there is a highly significant correlation between the effectiveness of the treatments, as represented by the average number of grubs per square foot for each treatment, and the yields of hay. Comparing yields of each treatment with the check it is seen that from 9 to 12 times as much forage was harvested from the treated plots, except for the toxaphene treatments where the yields were increased 3 to 6 times. The low yields in the untreated plots show the heavy losses in forage that may occur in meadows severely infested with larvae of the European chafer.

Analyses for residues of aldrin and dieldrin showed that the amounts ranged from 0.1 to 0.46 p.p.m. 5 days after treatment. In view of the short interval between treatment and sampling, these residues are not excessive and are, in general, less than residues obtained after spray or dust applications of equal amounts of aldrin and dieldrin (Hopkins 1951, Burrage 1951).

Table 4.—A comparison of several methods of applying dieldrin to control the larvae of the European chafer. Newark, New York. August 7, 1954.

Method	Formulation	Pounds Toxicant per Acre	Average No. Larvae per Sq. Ft.	Per Cent Reduction
Cyclone seeder	granulated	1	0.43	94.9
Cyclone seeder	granulated	2	0.14	98.4
Wheelbarrow seeder	granulated	2	0.46	94.5
Fertilizer spreader	granulated plus pyrophyllite	2	0.18	97.8
Low pressure sprayer	emulsion	2	0.87	89.8
Untreated			8.46	
L.S.D. at 1% level			1.89	

The data on residues remaining on different portions of the samples show practically no differences in amounts of toxicant between the *top*, *middle* and the *bottom* of the plants indicating that penetration of the dry foliage is nearly uniform and any residue that does persist probably consists largely of particles trapped in leaf sheaths and axils. In any event most of the toxicant penetrates the foliage canopy and falls on the ground as is desired.

As shown in table 4 granulated dieldrin, applied with a cyclone seeder at both rates of application, or with the wheelbarrow seeder, gave very good control of the grubs. The dieldrin granules mixed with pyrophyllite and applied with the fertilizer spreader also gave good control but the method is tedious and less practical. Dieldrin emulsion applied with a weed sprayer gave the least control of all the methods tried but still significantly reduced the grub population. The application of granular formulations of insecticides with cyclone and wheelbarrow seeders to control grubs in pastures and haylots situated on steep hillsides and other locations inaccessible to conventional machinery is apparently feasible and should prove to be a boon to farmers in the areas infested with European chafer.

SUMMARY.—Granulated formulations of aldrin, dieldrin, or heptachlor at 1 and 2 pounds per acre of actual toxicant, or chlordane at 10 pounds per acre gave excellent control of two successive generations of the larvae of the European chafer in a meadow. By controlling the grubs, these materials increased the yield of forage from 9 to 12 times. Toxaphene at the rates of 10 to 20 pounds of actual toxicant per acre, while unsatisfactory for the control of grubs at these rates still increased the yields of hay from 3 to 6 times.

An analysis for the residues of aldrin and dieldrin remaining on the hay show that only 0.1 to 0.46 p.p.m. remained 5 days after treatment. Such amounts would constitute no acute hazard to cattle and probably would be insignificant by harvest.

The cyclone and wheelbarrow seeders and hand-operated fertilizer spreader proved to be highly effective machines for the application of granulated insecticides. The weed sprayer, used for the application of an emulsion, was not as effective but still proved to be a useful and inexpensive machine for treating pastures and meadows. The hand-operated machines should prove useful to farmers whose pasture lands are on hills inaccessible to conventional power machinery.

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Automatic Cattle Sprayers for Fly Control in New Jersey[1]

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Interest was aroused when Bruce (1952) described an automatic cattle sprayer of his own design. The machine utilized a hydraulic pump to spray a small quantity of concentrated insecticide when cattle stepped on a hinged platform or treadle while going to or from the milking barn or to feed, water, or salt while in the pasture. Excellent control of biting flies (horn flies, horse flies, and stable flies) was noted.

Because the method appeared effective, it was tried under New Jersey conditions. A unit with one nozzle, which sprayed the back and one side of each cow as it passed over the treadle, was obtained and tried in a limited way in the summer of 1952. In 1953, a two-nozzle unit was obtained. This sprayed the top, one side, and legs of the cattle as they pased over the unit one way and treated the other side on the return trip. In 1954, 12 similar units were tested to determine not only the vagarities of the machines but also the effectiveness of various insecticide-repellent concentrates.

1952 TESTS.—Following the original recommendations, the automatic sprayer[4] was installed in a chute approximately 6 feet long and 2½ feet wide, just high enough for a cow to pass through. The sides and top were covered with burlap bags. This was installed in the pasture area so that a herd of 15 Hereford steers were treated as they went to water and returned to the pasture. Three or four trips were usually made each day. Counts of the flies on the animals were made twice during the morning and once in the afternoon. Horn flies, *Siphona irritans* (L.), predominated although large numbers of stable flies, *Stomoxys calcitrans* (L.), were prevalent during the last part of July and August. A few horse flies (*Tabanus* species) were also present.

The formulation (F-1) used in the sprayer was similar to the Pyrenone concentrate used by Bruce. It was found, as noted in table 1, that treated animals averaged 28 flies per count as compared to 75 prior to treatment.

1953 TESTS.—An improved two-nozzle sprayer[5] was started earlier in the season than in 1952, on a small herd of Hereford steers. When the treadle of the new unit was depressed, it actuated a 3-inch diameter air pump similar to one used for filling tires. This air blast pulled the liquid repellent out of two jars and forced it through specially designed nozzles, one placed about 48 inches above ground level and the other about 18 inches high. Each nozzle sprayed about 0.5 ml. per stroke of the pump. The chute arrangement was the same as the previous year except that the burlap bags were replaced by a canvas tarpaulin. The canvas cover was not damaged by the animals as were the bags, and it tended to cut down spray drift caused by wind.

Using this arrangement, excellent protection was obtained from a spray (F-10) containing 50% Crag Fly Repellent (a butoxypolypropylene glycol) and half the pyrethrins and synergist content of the formulation tried the previous year (F-1). Horn fly and stable fly annoyance was reduced from 85 flies per steer prior to treatment to 6 (Table 1). Good protection was also obtained from spray F-3 containing 1.7 per cent cyclethrin, a new synthetic pyrethrin-type chemical, and 10 per cent piperonyl butoxide plus emulsifier in oil. A formulation containing 40 per cent Crag Fly Repellent and 20 per cent Thanite[6] as the active ingredients was also found effective.

Two similar spray units were placed on dairy farms in Salem County in the southern part of the State. Unfortunately, actual fly counts were not available although excellent control was observed against mixed populations of horn and stable flies and a moderate infestation of horse flies, using the F-1 and F-3 formulations. The farmers were pleased with the effectiveness and ease of application and reported a boost in milk production of approximately 20 quarts from a normal total of about 120 quarts per day from each herd.

1954 TESTS.—As part of the experimental fly control program, 2-nozzle automatic sprayers of the same type used in 1953 were installed on 10 cooperating dairy farms in Salem County. These sprayers were installed in chutes covered with burlap bags, but the location of the chutes varied from one in a barn door to one in a field at a con-

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[4] A one-nozzle hydraulic pump sprayer, Z. & W. Machine Products, Inc., Cleveland, Ohio.

[5] Yellow Devil Automatic Cattle Sprayer, Engine Parts Manuf. Co., Cleveland, Ohio.

[6] Commercial isobornyl thiocyanoacetate.

Table 1.—Summary of tests made with automatic cattle sprayers in New Jersey.

PER CENT ACTIVE INGREDIENTS[c]	FARM NUMBER	DATE	AVERAGE NUMBER OF BITING FLIES[a] PER ANIMAL[b] PER COUNT	
			Untreated	Treated
		1952	*One Spray Nozzle*	
F-1				
1.0 pyrethrins	New Brunswick, 13	7/31–8/22	71	28
10.0 piperonyl butoxide				
15 emulsifier				
		1953	*Two Spray Nozzles*	
F-10				
50 Crag F. R.	New Brunswick, 13	7/2 –7/17	85	6
0.5 pyrethrins				
5.0 piperonyl butoxide				
15 emulsifier				
F-3				
1.7 cyclethrin	New Brunswick, 13	7/20–8/10	45	6
10 piperonyl butoxide				
15 emulsifier				
ECT				
40 Crag	New Brunswick, 13	8/11–8/24	23	7
20 Thanite				
15 emulsifier				
		1954		
F-1C				
1 pyrethrins	Salem, 43	8/2 –8/27	19	4
10 piperonyl butoxide				
10 emulsifier				
AT-20				
0.5 pyrethrins	Salem, 41	6/24–7/16	160	4
5.0 piperonyl butoxide	Salem, 27	7/14–8/6	49	3
0 emulsifier				
AT-20 WE				
0.5 pyrethrins	Salem, 28	6/7 –7/1	75	2
5.0 piperonyl butoxide				
10 emulsifier				
AT-0				
0.2 pyrethrins	Salem, 29	6/7 –6/18	23	6
2.0 piperonyl butoxide				
F-10C WOE				
50 Crag	Salem, 42[d]	6/24–7/7	85	10
0.5 pyrethrins				
5.0 piperonyl butoxide				
AT-4				
50 Crag	Salem, 35	8/4 –8/20	22	6
0.2 pyrethrins				
2.0 piperonyl butoxide				
AT-24				
50 Crag	Salem, 27	8/11–8/27	15	7
0.2 cyclethrin				
2.0 piperonyl butoxide				
AT-7				
50 Crag	Salem, 42[d]	8/18–8/27	55	10
5 methoxychlor				
15 velsicol				
AT-8				
50 Crag	New Brunswick, 13	7/14–8/5	12	5
10 Thanite				
AT-8L				
50 Crag	New Brunswick, 13	8/10–8/20	13	6
10 Lethane				

(*Continued on next page*)

(Table 1.—Continued)

Per Cent Active Ingredients[c]	Farm Number	Date	Average Number of Biting Flies[a] per Animal[b] per Count	
			Untreated	Treated
AT-21				
0.5 cyclethrin 5.0 piperonyl butoxide	Salem, 35	8/23–8/27	55	2
AT-Oil				
0	Salem, 41	6/8–6/24	124	87
Cattle not sprayed on nearby farms:				
	Salem, 37	July, 1954	15	
		August, 1954	25	
	Salem, 36	July, 1954	90	
		August, 1954	119	

[a] Horn flies, prevalent all season with stable flies, becoming abundant in late July and continuing for rest of summer. Horse flies were relatively light in all years.
[b] At New Brunswick farm Hereford steers were treated. At Salem farms, various breeds were sprayed including Holstein, Guernsey and Jersey.
[c] Applied in petroleum distillate.
[d] One trip per day through double unit. All other locations two to four trips per day.

siderable distance from the barn. In some instances, cows had more or less free access to the sprayer and could go back and forth at will as compared to two installations in which only one round trip occurred per day.

Exceptionally dry weather during June and July probably reduced the number of biting flies, especially *Tabanus* species normally present in this area. Horn flies were prevalent throughout the season; stable flies became abundant in late July and continued until observations ceased in September. Although the farms were relatively close to each other, considerable variation was noticed in the fly population from farm to farm.

A number of different formulations were tried, and the results of fly counts on untreated cows prior to the use of each mixture and during its use are summarized in table 1. Due to the wide range in conditions encountered at each location and the varying numbers of flies prior to treatment, calculation of per cent repellency based on counts on untreated animals would be misleading; effectiveness of treatments can be judged on the basis of the average number of flies per cow per count. Counts of less than 5 flies were considered excellent, from 5 to 10 good to fair and above this proportionately poor. Because of the varied conditions, direct comparisons between most of the treatments are also considered inadvisable. Nevertheless, general trends are evident and can be summarized.

Synergized pyrethrins usually provided satisfactory protection. These tests indicate that under the conditions encountered in 1954, the concentration of pyrethrins and synergist could be lowered from that used previously. For instance, 0.5 per cent pyrethrins with 5.0 per cent piperonyl butoxide (AT-20) reduced flies to four or less per cow. Even a formulation containing 0.2 per cent pyrethrins and 2 per cent piperonyl butoxide reduced flies to only six per cow (AT-0).

There was no clear cut indication that use of an emulsifier in the concentrate affected repellency but inclusion of an emulsifier will also provide a product which can be diluted with water for application by conventional means. On the other hand, the product containing an emulsifier might become too viscous for satisfactory spraying as a concentrate. It might also tend to wash off the animals more easily in wet weather.

At Salem Farm 42,[7] farm management was such that the herd could make only one round trip each day. To provide coverage on both sides of the cows, two units were placed in one long chute, With one trip, equivalent to two trips through the usual sprayer arrangment, reduction in fly population was still considerable. From an original population of 55 to 85 flies per cow, use of a formulation containing Crag Fly Repellent and synergized pyrethrins (F-10C WOE) or one containing Crag Fly Repellent and methoxychlor (AT-7) reduced the number to 10 flies per cow.

Cyclethrin appeared to behave similar to synergized pyrethrins. When used at 0.5% with 5% piperonyl butoxide, only two flies per cow were noted.

Two isothiocyanate-type chemicals, Thanite and Lethane (AT-8 and AT-8L), were tried at different times at New Brunswick Farm 13 under relatively light fly nuisance conditions. When combined with Crag Fly Repellent, reduction was about the same as was experienced in 1953 with a Crag and Thanite formulation. No skin injury was noticed from these thiocyanate formulations under the conditions of the tests.

An indication that the insecticide-repellent formulations were responsible for the reduction in flies noted on the cows was provided by the figures obtained when the base oil (Bayol D) alone was used in the spray machine. The average number of flies on the cows during the oil treatment remained at about 90 per animal. With a pyrethrins formulation, such as AT-20 already mentioned, flies were reduced to only four per animal. Another indication of the effectiveness of the machine was the fly counts obtained on two herds in the Salem area which were not treated. On Farm 37, the average number of

[7] Farm locations have been designated by numbers.

flies per cow during July was 15; during August it was 25. On Farm 11, the average number of flies per cow during July was 90; in August it was 119.

The dry weather induced a number of farmers to supplement pasture feeding. This complicated any correlation between reduction in flies and improvement in milk production so that no consistent trend was evident.

COSTS AND OTHER FACTORS.—Automatic sprayers may be more economical to use than other spray methods for controlling flies on cattle. The initial cost of the two-nozzle machine was about $45.00, to which must be added the cost of the chute to house it. This cost would probably approximate the amount spent on a good electric sprayer used for direct application of sprays to the animals. Based on cost of ingredients, a formulation such as F-1C, containing 1.0 per cent pyrethrins and 10% piperonyl butoxide in an emulsifiable oil, used in an automatic sprayer 4 times per day (2 round trips) would cost about $13.25 for a month's supply for a herd of 50 cattle. The same formulation diluted 1 to 9 with water applied at the rate of 1 pint per animal every 4 days would cost about $39.00 for the month. The ingredient cost of a dilute oil spray containing 0.025 per cent pyrethrins and 0.25 per cent piperonyl butoxide applied daily at a rate of 2 ounces per animal would cost about the same as the automatic sprayer formulation, i.e. about $12 to $13. However, the consumer cost of the dilute oil spray would probably be 3 to 4 times the ingredient cost while the consumer cost of the concentrate would probably be only about twice the ingredient cost. In addition, the daily chore of spraying the animals must be considered.

The balance in favor of the automatic sprayer is even more pronounced if sprays such as AT-20 containing half the pyrethrins or piperonyl butoxide content or F-10C containing Crag Fly Repellent and synergized pyrethrins are considered. Ingredient cost of such sprays varies from $6.00 to $9.00. Sprays containing other ingredients such as Crag Fly Repellent and methoxychlor or Thanite are even cheaper.

As with many other devices, these machines are not trouble-free. Persistent horn fly and stable fly annoyance was often noted on the legs and under line. This appeared to be due not so much from ineffectiveness of the spray ingredients as from occasional deficiencies in the machine. Some of this trouble could be remedied by easier adjustment of the nozzles. Another desirable feature, if it is possible with the present pump mechanism, would be the addition of an extension arm and nozzle so that both sides of the animal could be treated at one time. The use of two units in one chute accomplishes this, but it is a cumbersome and expensive expedient.

Experience has revealed ways of installing and using these machines to obtain the best results. An initial piecemeal installation is recommended so that animals will become accustomed to it and will not balk at going through the chute. The treadle may be installed in a non-operating position on one day, followed on succeeding days by the upright and the chute side-boards. Finally the chute can be covered and the sprayer started. Installed in this way, the normal curiosity of the cows was satisfied and their timidity overcome.

To prevent cows stepping along the side of the treadle rather than on it, the chute should be just wide enough to hold the treadle mechanism. If possible, the chute should be placed so that prevailing winds do not sweep through. Installation in unprotected barn doors may not be satisfactory for this reason.

To get adequate coverage that will persist during the day, at least two round trips over the machine are desirable. Best results were obtained where three or four round trips occurred.

In one instance, lime used for control of foot-rot was placed in a depression just ahead of the machine. The cows carried this on to the treadle and soon clogged it. This could probably have been prevented by placing the foot treatment a little farther from the machine.

Daily inspection of the machines operation is desirable. Although fairly sturdy and reliable, constant exposure to animal usage and the weather makes some maintenance work necessary. The nozzles may need an occassional cleaning or adjustment. In several instances, rivets holding the spray jar attachment became loose, making it necessary to replace them with bolts. The machines should be dismantled and stored in a dry place during the winter; worn parts should be replaced at this time.

The newer residual treatments, baits, and space sprays used in the barn may control annoyance from house flies and other flies which are indoors. This may tend to cause the farmer to overlook the fact that serious reduction in milk and meat production can occur from biting flies while the cows are in the field. When used properly, the automatic sprayers can be an effective labor-saving means that provide the necessary protection.

SUMMARY AND CONCLUSIONS.—The automatic spray units tried in New Jersey utilized the principle of applying a small quantity of a concentrate spray, preferably two to four times per day. Varying concentrations of synergized pyrethrins in petroleum distillate were found to be effective; a typical formulation contained 0.5 per cent pyrethrins and 5 per cent piperonyl butoxide. To improve effectiveness and reduce cost, various other ingredients were tried or substituted for all or part of the synergized pyrethrins. These ingredients included Crag Fly Repellent, cyclethrin, methoxychlor, Thanite and Lethane. The machines should be installed initially a part at a time over a period of several days in order to facilitate cow acceptance. Experience over a 3-year period with the automatic spraying machine indicates that when properly installed, maintained, and provided with an effective spray formulation, this labor-saving device can be a useful supplement to other fly control practices.

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Effect on the Oriental Fruit Moth of Parathion and EPN Applied to Control the Plum Curculio on Peach[1]

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Studies of the effect on the oriental fruit moth, *Grapholitha molesta* (Busck), of parathion and EPN sprays applied for control of the plum curculio, *Conotrachelus nenuphar* (Hbst.), were undertaken in peach orchards near Moorestown, N. J., in 1950. Their purpose was to show the extent to which the spray applications reduced overwintering-brood adults and first-brood larvae of the oriental fruit moth and how much this reduction affected the population of succeeding broods up to harvest time of Summercrest and Elberta varieties. Observations made in 1950 and 1951 have already been reported (Brunson 1952). Those made in 1952 and 1953 are presented herein.

In the vicinity of Moorestown, moths of the overwintering brood appear in the orchards during the latter part of April and are present until about June 15 (based on observations during 1946 to 1953). First-brood larvae appear early in May and are present until about June 20. The insecticides were applied against the plum curculio during May and the first few days of June; therefore, any stages of the oriental fruit moth present in the orchards during this period were subjected to them. First-brood moths appear about June 15 and are present until about July 15. Second-brood larvae are present from about June 20 to July 20, and moths from about July 15 to the latter part of August. The third brood begins about July 20 and continues until the end of the commercial peach growing season. Most over-wintering larvae are of the third, fourth, and partial fifth broods (Peterson & Haeussler 1930).

Materials and Methods.—Parathion was used at the rate of 1.5 pounds of 15 per cent wettable powder and EPN at the rate of 1.5 pounds of 25 per cent wettable powder per 100 gallons. The spray schedule consisted of applications at the time of shuck split and approximately 10 and 20 days thereafter. The second application after shuck split was made in the experimental orchards, since some moths of the overwintering brood are still present on June 1. The schedule used for control of the plum curculio in the check orchards consisted of an application of lead arsenate at shuck split and another 10 days later. This insecticide has no appreciable effect on the oriental fruit moth. Sulfur was included in all applications in the experimental and check orchards.

Parathion was applied in three and EPN in two experimental orchards each year. The same experimental orchards were used in both years except one of those sprayed with parathion. All the experimental and check orchards contained Summercrest or Elberta peaches and most of them contained other earlier maturing varieties. In 1953 the third application was not made in two parathion-sprayed orchards owing to unfavorable weather. Speed-type sprayers were used in the experimental orchards, and both sides of each row of trees were sprayed throughout the orchards on each spray date. Speed-type sprayers and hand guns were used in the check orchards. Cooperating growers applied the parathion and members of the station staff the EPN sprays.

The moth populations in the orchards were estimated by means of bait traps. The bait consisted of 10 per cent sugar (No. 10) solution in water containing 0.5 ml. of a stock attractant solution (terpinyl acetate, extra grade, 97 parts and Tween 20, 3 parts) per quart. Traps consisted of 1-quart paraffin-dipped cylindrical ice cream cartons covered by 4-mesh hardware cloth.

Moth Abundance.—Six traps were hung under foliage near the top of alternate trees in one row in each orchard. Baits were renewed at 2-week intervals, and old and new baits were provided by renewing the bait in alternate traps each week. Traps were placed in the orchards on April 30 in 1952 and April 22 in 1953, at least a week before the first spray was applied, and were examined for moths on Monday, Wednesday, and Friday of each week until the later part of July. The numbers of moths caught each week are shown in tables 1 and 2.

Moths were present in considerable numbers in all the orchards before the first application of insecticide. The effect of parathion and EPN on the moth population is indicated by a comparison of the average numbers caught in the experimental and check orchards (1) before the first spray was applied, (2) between the first application and June 15, and (3) after June 15. During these periods the average numbers caught in the experimental orchards were 65, 88, and 89 per cent, respectively, fewer than the number caught in the check orchards in 1952 and 25, 75 and 95 per cent fewer in 1953.

Larval Population in Twigs.—Succulent twigs, preferred by larvae for feeding, are abundant in orchards near Moorestown from about the middle of May to the last week of July, depending on weather conditions. When succulent twigs become scarce, the larvae feed in fruit. Twigs that can be used for food become scarce near the beginning of third brood. Since the peak of third-brood larval abundance occurs about the middle of August, fruits of varieties that ripen before this time are not usually seriously injured.

An estimate of the larval population was obtained by collecting infested twigs each week from mid-May to mid-July from 10 sample trees about the same distance apart along the periphery of each experimental and check orchard. The number of infested twigs collected (Tables 1 and 2) indicated the population of larvae that survived the insecticides in May or early June and the progeny of survivors thereafter.

The larval population was low in the experimental orchards. Since moths were caught in these orchards during the periods that sprays were applied, it is indicated that only a few of their progeny survived. Before June 20, 98 per cent fewer infested twigs were collected in the experimental orchards than in the check orchards each

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Table 1.—Average number of oriental fruit moths captured in traps and infested twigs collected from orchards sprayed with parathion, EPN, or lead arsenate in 1952.[a]

Period	Moths Captured in 6 Traps in Orchards Sprayed with—			Infested Twigs from 10 Sample Trees in Orchards Sprayed with—		
	Parathion	EPN	Lead Arsenate (Check)	Parathion	EPN	Lead Arsenate (Check)
April 30–May 2	7	4	28	—	—	—
May 3–9	72	46	158	—	—	—
10–16	32	23	216	0	0	1
17–23	5	4	33	0	0	8
24–30	11	6	54	0.3	0	36
May 31–June 6	4	3	40	0.3	0	32
June 7–13	6	2	103	1	0	54
14–20	5	4	135	2	0.5	22
21–27	7	2	80	2	0	48
June 28–July 4	24	2	200	0.3	0.5	49
July 5–11	36	5	178	2	0	60
12–18	45	6	102	6	0	35
19–24	—	—	—	9	0.5	20
Total	254	107	1327	22.9	1.5	365
Before first spray	77	41	179	—	—	—
Between first spray and June 15 (moths) or June 20 (infested twigs)	59	47	453	4	0.5	153
After June 15 (moths) or June 20 (infested twigs)	118	19	695	19	1	212

[a] Three orchards were sprayed with parathion, two with EPN, and three with lead arsenate. Parathion and EPN applied on May 5–8, 16–19, and May 27–June 4.

Table 2.—Average number of oriental fruit moths captured in traps and infested twigs collected from orchards sprayed with parathion, EPN, or lead arsenate in 1953.[a]

Period	Moths Captured in 6 Traps in Orchards Sprayed with—			Infested Twigs from 10 Sample Trees in Orchard Sprayed with—		
	Parathion	EPN	Lead Arsenate (Check)	Parathion	EPN	Lead Arsenate (Check)
April 22–24	13	2	11	—	—	—
April 25–May 1	63	16	55	—	—	—
May 2–8	73	37	109	—	—	—
9–15	156	24	327	0	0	1
16–22	40	15	153	1	0	37
23–29	3	2	40	0	0	35
May 30–June 9	3	2	20	0	0	30
June 6–12	2	3	29	0	0	19
13–19	3	1	61	3	0	10
20–26	30	2	326	0	0	6
June 27–July 3	35	3	422	3	0	73
July 4–10	27	2	142	7	2	150
11–17	10	5	31	1	1	36
18–24	18	6	40	1	0	6
Total	476	120	1766	16	3	403
Before first spray	127	54	131	—	—	—
Between first spray and June 15 (moths) or June 20 (infested twigs)	226	47	619	4	0	132
After June 15 (moths) or June 20 (infested twigs)	123	19	1016	12	3	271

[a] Three orchards were sprayed with paration, two with EPN, and three with lead arsenate. Parathion and EPN applied on May 4–8, 19–21, and 29.

year and after that date 94 per cent fewer in 1952 and 97 per cent in 1953.

RIPE-FRUIT INJURY.—The larval injury in ripe Summercrest and Elberta fruits was determined on 400 fruits from each sprayed orchard. The average fruit-sampling date was August 18 in 1952 and August 17 in 1953. Samples were obtained by taking one fruit at random from each tree passed while crossing the blocks of trees several times in different directions. Each fruit was dissected and examined for larvae or signs of larval feeding. The results are shown in table 3.

The low degree of ripe-fruit injury in the experimental orchards indicated that the oriental fruit moth population remained low through the first half of August. The average injury in these orchards was 86 per cent less in 1952 and 89 per cent less in 1953 than in the check orchards. These data compare favorably with the percentage difference in the number of infested twigs collected from experimental and check orchards during the second brood and indicate that few moths migrated into the experimental orchards from distant sources during the third brood.

In 1952 one parathion-sprayed orchard adjoined a small unsprayed block of 2-year-old peach trees. The number of moths caught in this orchard after June 15 was 179, the number of infested twigs collected after June 20 was 28, and ripe-fruit injury was 9.3 per cent, as compared with averages of 53 moths, 8 infested twigs, and 1.8 per cent ripe-fruit injury in the other experimental orchards. In 1953 one parathion-sprayed orchard was not sprayed properly despite the fact that the machine used had ample capacity. The number of moths caught in this orchard after June 15 was 299, the number of infested twigs collected after June 20 was 32, and ripe-fruit injury was 6.8 per cent, as compared with averages of 27 moths, 2 infested twigs, and 0.7 per cent ripe-fruit injury in the other experimental orchards.

Observations during 4 years on the effect of parathion and EPN on the oriental fruit moth applied to control plum curculio have shown that, if the early-season curculio schedule is to be relied on for controlling the oriental fruit moth without preharvest sprays, orchards must be sprayed thoroughly and consideration must be given to possible nearby sources of reinfestation, such as unsprayed young peach trees, peach orchards not sprayed with phosphorus insecticides for simultaneous control of plum curculio and oriental fruit moth, and neglected peach and apple orchards.

If spray applications are not thorough and if there are possible sources of reinfestation nearby, at least one more

Table 3.—Per cent of peach fruits injured near harvest in orchards sprayed with parathion, EPN, or lead arsenate, 1952 and 1953.

Treatment	Orchard Number	1952		1953	
		Variety	Fruit Injured	Variety	Fruit Injured
Parathion	1	Elberta	9.3	Elberta	0.3
	2	Summercrest	0.8	Elberta	6.8
	3	Summercrest	2.8	Summercrest	1.0
		Average	4.3	Average	2.7
EPN	4	Elberta	1.8	Elberta	0.3
	5	Elberta	1.8	Elberta	1.3
		Average	1.8	Average	0.8
Lead arsenate (check)	6	Elberta	22.0	Summercrest	13.8
	7	Elberta	30.0	Summercrest	21.5
	8	Summercrest	21.0	Summercrest	15.0
	9	Summercrest	37.8	Elberta	31.0
	10	Summercrest	16.5	Elberta	6.5
	11	Summercrest	18.8	—	—
		Average	24.4	Average	17.6

application should be made about 3 weeks before harvest time of Summercrest, Elberta, or other varieties that ripen the latter part of August.

SUMMARY.—Studies in peach orchards near Moorestown, N. J., of the effect on the oriental fruit moth, *Grapholitha molesta* (Busck), of parathion and EPN sprays applied to control the plum curculio, *Conotrachelus nenuphar* (Hbst.), were continued in 1952 and 1953. As compared with the orchards sprayed with lead arsenate, in orchards receiving these sprays there were reductions of 80 per cent of the overwintering-brood moths and 90 per cent of the first-brood moths caught in traps, 95 per cent of the twigs infested with the first and second brood, and 90 per cent in ripe-fruit injury.

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Cooperative Control of the Desert Locust and Other Major Pests in the Middle East and South Asia[1]

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One of the many kinds of technical assistance provided in our foreign programs, plant pest control is an effective and economical means of helping people overcome fear and discontent in their struggle to build a better agriculture. While technical assistance programs have been greatly expanded in recent years, we have been brought face to face with insect problems entirely new or little known to us. Outstanding among these is the desert locust, *Schistocerca gregaria* Forskal. From Biblical times nations of the Middle East, South Asia, and Africa have suffered one locust plague after another. In our short experience in providing assistance to nations affected by the locust we have seen a change in their attitude and willingness to cooperate in programs of national and international significance. In the overall program of technical assistance, with the exception of locust control, plant pest control still plays a minor role.

THE REGIONAL LOCUST CONTROL PROGRAM.—The Regional Locust Control Program, which was conducted by the former Bureau of Entomology and Plant Quarantine in cooperation with the Technical Cooperation Administration of the Department of State from November 1951 to June 1954, was an outgrowth of the successful operation undertaken by the latter organization in the spring and summer of 1951. In that year the Government of Iran requested aid from the United States in combating one of its worst locust invasions in 80 years. The assistance given to Iran in the form of spray planes, insecticides, and technicians paved the way for close cooperation in many other agricultural endeavors. It helped stem a serious locust threat to thousands of acres of croplands. It gained confidence of both farmers and government officials and stimulated the people of Iran and nearby countries to develop more effective control organizations. In the same season the United States extended its aid in locust control to Pakistan and India following requests to the Department of State from their governments.

In 1952 the Regional Locust Control Program was enlarged to provide for regional operations under bilateral agreements with nations of the Middle East, South Asia, and Africa at the request of cooperating nations. The operations were coordinated under a field office located at Beirut, Lebanon, and supervised by the Department of Agriculture under authority delegated by the Technical Cooperation Administration. In addition to the program director there was a staff of four entomologists and one administrative assistant. In the fiscal year 1952–53 nine Piper Cub airplanes, equipped with the necessary instruments, spraying gear, and spare parts, were purchased and placed at the disposal of the program director. The planes were operated by pilots and mechanics provided for under a commercial contract. They usually operated in units of three, with an entomologist supervisor over each unit. The program director was authorized to dispatch them to any country requesting aid and with whom agreements to meet locust control outbreaks had been signed.

FAO TECHNICAL ADVISORY COMMITTEE.—The Food and Agriculture Organization (FAO) of the United Nations, realizing the need for stronger coordinated organization between countries, called a conference on locust control in October 1951. Delegates from 13 nations met in Rome. Since that time FAO has called one or more conferences each year somewhere in the Middle East or in Rome. Plans of national campaigns are submitted for review and recommendations made for means of providing supplies to countries in need of them. Following the first conference in 1951, the FAO council established a technical advisory committee on locust control. On this committee are experts from Egypt, France, India, Iran, Pakistan, the United Kingdom and the United States. This committee has been meeting once or twice each year to appraise the locust situation, discuss strategy, indicate priority of infested areas, and advise the FAO on policy. FAO's new role in locust control has been effective in bringing all interested countries together for joint acceptance of a continued international campaign against the desert locust. Other programs conducted by the FAO in many countries affected by locusts have facilitated entry of its technicians and officers and have assisted in bringing about cooperative action more promptly than could any single government.

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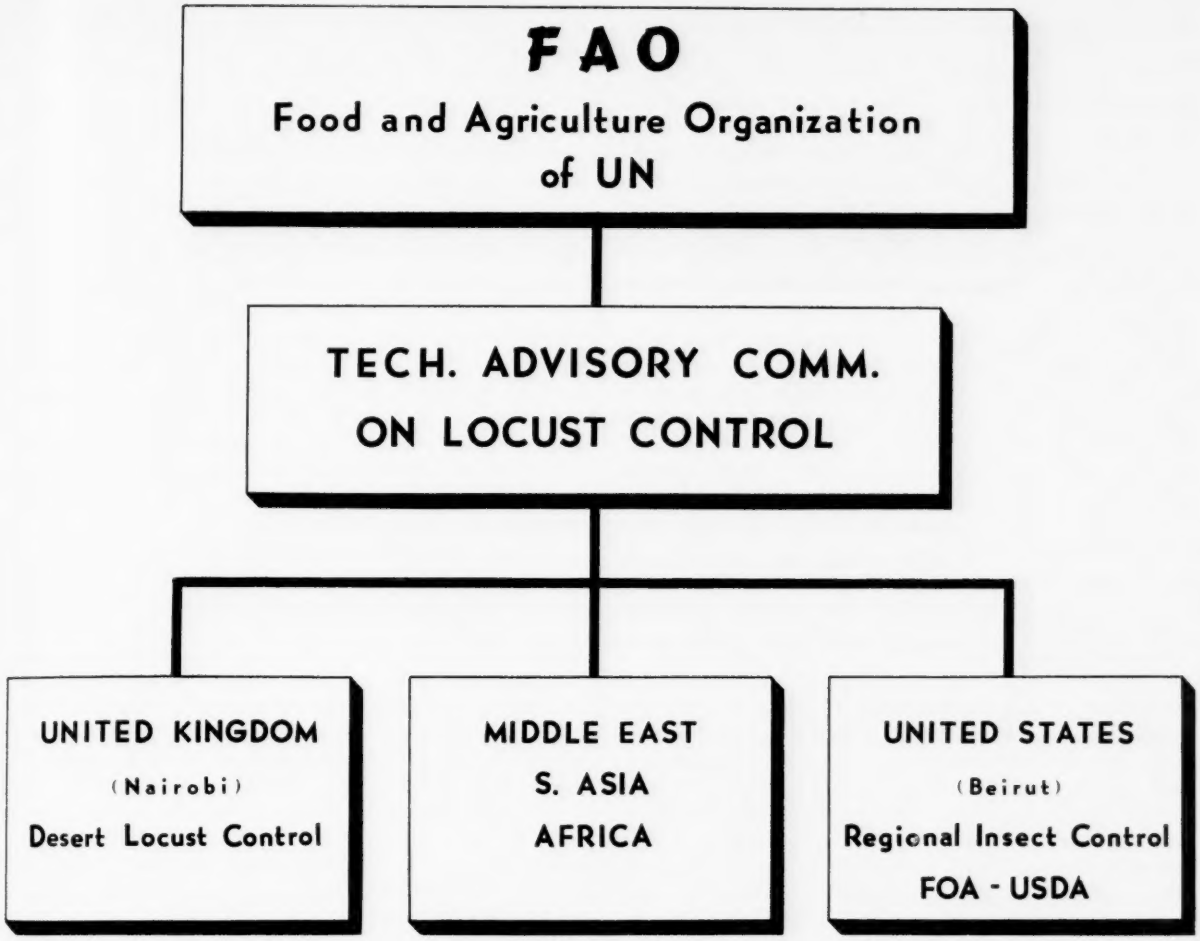

FIG. 1.—Organizational chart showing relation of FAO Technical Advisory Committee to countries participating in international locust control.

In the 1952–53 campaigns FAO provided $500,000 for the purchase of trucks, sprayers, airplanes and insecticides to member nations in the threatened areas.

INTERNATIONAL ORGANIZATION OF LOCUST CONTROL.—The organization of international locust control is not easily described. Under FAO and its advisory committee on locust control are three blocks of one or more countries, each working in its own way and with its own resources but following an overall plan that is agreed to in advance. In the first block (Fig. 1) are the United Kingdom and its dependencies, together with some countries of East Africa bordering upon the Red Sea. The United Kingdom, through the Desert Locust Survey and Control Organizations, both headquartered at Nairobi, Kenya, has borne the brunt of all locust control in East Africa and the Arabian peninsula for many years. Staffed with approximately 150 technical and administrative personnel, and operating in 11 countries, these two organizations are due major credit for the present-day concept and policy on the desert locust and its control. Forecasts emanating from the British Anti-locust Research Centre in London are based largely on reports from their officers. These United Kingdom organizations are sympathetic to FAO's role in locust control and consider it of value in planning toward more permanent organization. British experience has also been invaluable to FAO and all afflicted countries.

In the second block are 20 or more countries of the Middle East, South Asia, and Africa, each with its own locust organization working independently or collectively. The bulk of actual locust control is conducted by the governments of these countries at their own expense and without outside aid. Approximately $12,000,000 is expended annually by all governments for locust control. Eighty-five per cent of this amount is borne by the countries in these areas.

OBJECTIVES OF U. S. PARTICIPATION.—The United States participation is represented in block three. The objectives of our activities have been (1) to assist the countries of the Middle East, South Asia, and Africa in the control of the desert locust through the demonstration of aerial spraying techniques and reconnaissance as a supplement to other control measures, (2) to assist in the training of native pilots and mechanics to provide for the continuation of aerial spraying, and in the training of local entomologists in field survey and control meth-

ods, (3) to give guidance and assistance in the coordination and execution of aerial and ground control activities, and (4) to promote international cooperation by working with technicians and authorities of individual nations and agencies concerned with the control of the locust.

Our regional program is financed from Mutual Security Act funds under a "special project" agreement between the Foreign Operations Administration and the Pest Control Branch of the Agricultural Research Service in the Department of Agriculture. It has served Iran, Iraq, Pakistan, India, Jordan, Ethiopia, Lebanon, Saudi Arabia, and Libya. Spray demonstrations have been conducted in six of these countries. Agreements with countries are for 1 year, but are extended or amended according to the needs and availability of funds. Each bilateral agreement provides that a host country furnish (1) ground support for aerial spraying, gas and oil for the aircraft, and assistance in their maintenance; (2) transporation of insecticides, fuel, water, and other supplies; (3) lodging and meals for U. S. personnel when not otherwise available; (4) trucks, jeeps, and hundreds of laborers; (5) insecticides and bran for baiting in ground control; and (6) technicians to supervise their own country operations and cooperate with the regional control personnel.

CONTROL MEASURES.—Prior to the establishment of the Program, control operations throughout the locust-infested region were confined almost exclusively to ground applications of poison baits. BHC is still the most popular and widely used poison ingredient. It is mixed with wheat, barley, or rice bran. Poison bait is directed mainly against bands of young hoppers at the rate of 10 to 20 pounds per acre, the equivalent of 0.1 to 0.2 ounces of gamma isomer per acre. Wet baits have consistently given better results than dry baits in semidesert countries. In some more humid areas, such as parts of India, BHC dusts are applied with power machines and hand dusters. Spraying has recently become more popular and may replace dusting. Liquid BHC and dinitro-orthocresol are also used as sprays, particularly in the British protectorates.

Control operations conducted on an individual country basis have not proved entirely satisfactory because of the vast and inaccessible areas inhabited by locusts and the difficulties of transportation. The Regional Locust Control unit has supplemented the efforts of various national and international agencies, including the Food and Agriculture Organization, particularly where time and speed of application were important. It has added aerial reconnaissance and spraying, which not only have increased the effectiveness of the ground operations but have added a distinct control measure which has met the approval of governments wherever it has been demonstrated. It has also stimulated the coordination of efforts of the nations within whose borders locusts must be combated, and has contributed to progress in international cooperation by these nations. In our first effort to attack the desert locust in 1951, a 60 per cent aldrin solution was used in kerosene for aerial spraying at the rate of 2 to 3 ounces of actual aldrin per acre. At the suggestion of Pakistan authorities water was later substituted for kerosene. The effects were equally as good. This proved to be quite an innovation since it had previously been considered that the concentrated material could not be used successfully in an aqueous solution. It also meant a considerable economy in the cost and transportation of the diluent. Crystallization of the 60 per cent aldrin stock material in the drums as imported necessitated redissolving. This difficulty has been largely overcome since 1952 by use of a 40 per cent emulsifiable concentrate in either kerosene or water.

U. S. INSECTICIDES GAIN PROMINENCE.—The use of this American product overseas has aroused interest in many of the other chlorinated hydrocarbons as well as the organic phosphorus compounds for the control of locusts and many other insects. Entomologists in Pakistan, Iraq, and elsewhere have learned that locusts can be controlled with aldrin in baits more economically than with BHC. The superior residual effect of the newer insecticides will undoubtedly be a factor in their acceptance as poisons for the desert locust. In trials conducted in East Africa, dieldrin applied at the rate of 2 ounces per acre on egg beds up to 14 days before hatching, gave almost 100 per cent control of the resulting first-instar hoppers.

As more emphasis was placed on the locust problem, it became apparent that the efforts of an individual country were not enough. Locusts migrate from country to country, often flying hundreds of miles; they do not respect national boundaries. The bringing of nations together to exchange scientific data and to reach agreements on principles has created a new sense of responsibility in international locust control. The free exchange of control teams, supplies, and equipment between countries has encouraged confidence and belief that this age-old problem can be dealt with to advantage when done so collectively and in the proper spirit. The combined efforts, while not perfect, have prevented crop losses from the desert locust throughout the Middle East and Southern Asia for the last 4 years.

ROLE OF USDA IN THE FOA PROGRAM.—The participation of the Department of Agriculture in technical assistance programs which are now carried out under the direction of the Foreign Operation Administration is based on a memorandum of agreement dated February 18, 1954. In this memorandum authority was granted to the Department in connection with the planning and implementation of insect-control programs in cooperation with governments of the Near East, South Asia, and Africa. On the basis of this authority a special project was agreed upon between the Agricultural Research Service and the FOA and made effective July 1, 1954.

Within the ARS the Plant Pest Control Branch is now the agency responsible for operating the Regional Insect Control Project. This project supersedes the former Regional Locust Control program, but many of its responsibilities and features remain the same. It assists the U. S. Operations Missions and the governments of cooperating countries in the development and operation of practical control programs against major insect pests. Insect-control activities of FOA that involve cooperation with international and other organizations are also receiving special attention, particularly in relation to locust control.

The work of the Plant Pest Control Branch is designed to fulfill commitments to countries under the former Regional Locust Control Program with respect to the training of pilots, the evaluation of locust problems, and the

demonstration of control operations, where necessary, to meet outbreaks that threaten the agriculture of countries requesting assistance. Major insect problems are being explored with respect to their adaptation to control operations with hand and engine-powered ground equipment and aircraft. Technical guidance is being given to biological control measures in Iran. Every effort is made to develop leadership among the local technicians in applied entomology through on-the-job training.

In the planning, execution, and operation of these programs, close cooperation is maintained between the director of the U. S. Operations Mission in each country and the coordinator of the Regional Project at Beirut. Their responsibilities have been carefully outlined and agreed upon. The coordinator is William B. Mabee, formerly of the grasshopper control project in this country. He acts under the direct authority of the chief of the Plant Pest Control Branch, and is responsible to the Branch for the conduct of the program in all its technical aspects. Liaison between the specialists assigned to this project and the research agencies of the Department provides the technical information on which they base their programs. We now have a staff of 15, including pilots, stationed in six countries, namely, Lebanon, Jordan, Iraq, Iran, Pakistan, and Ethiopia.

RESULTS OF COOPERATIVE CONTROL PROGRAMS.—In the short period these insect-control programs have been underway progress has been noteworthy. Approximately 250,000 acres have been sprayed from the air for the control of locusts in these six countries during the last four seasons. This amount of spraying protected food crops and pastures in more than 2,500,000 acres. Over 10,000 acres were sprayed for control of rice, sugar cane, and cotton insects. Several dozen pilots trained to operate and maintain spray planes have participated in control programs. Most countries have already purchased or contemplate purchasing their own planes for insect control.

Plant protection organizations have taken a greater interest in the control of locusts and other insects, and have consequently received bigger appropriations from governments for research and control. The success of the programs has brought hope to the people and encouraged them to increase their plantings. The efforts of the various nations have been better coordinated, a factor that has contributed to progress in international cooperation.

These efforts have made it possible for the personnel attached to the project to direct their activities to insect problems other than locusts. The olive fly, sen pest of wheat, pyrilla of sugar cane, bollworms of cotton, rice borers, and the like are now being investigated. Importation of new insecticides is on the increase. Large-scale field demonstrations are under way with many chemicals heretofore little known or untried in most countries.

BENEFITS TO THE UNITED STATES.—In addition to the benefits that cooperating countries are deriving from these programs, the United States also has a direct interest in their successful operation. Never before in the 100 years that entomology had been recognized as a profession in the United States have we been so conscious of the need to know more about foreign insect problems as they affect us either directly or indirectly. We believe that every effort expended toward increasing our knowledge of insect enemies that may sometime reach this country is worth while. The European corn borer, the Japanese beetle, and the Mexican boll weevil are only a few examples. We found it advisable to send entomologists abroad to the native homes of these species to learn more about them and their natural enemies. We have been threatened with such pests as the citrus blackfly, the oriental fruit fly, and more recently the Khapra beetle. We can appreciate their significance if left unchecked and all that is involved in obtaining funds and cooperation for research and control purposes to hold them in abeyance.

First-hand information on foreign pests obtained abroad can also be helpful toward improving foreign trade relations with the United States. Entomologists of government, land-grant colleges, and industry have already played an important role in this connection. Their work abroad has exerted a noticeable influence on the export trade of our pesticides and farm equipment. Through better understanding and familiarity with these products, foreign pest-control authorities are in position to service a country's needs more satisfactorily.

The future appears brighter for the peoples of these under-developed areas. Through better pest control they are blazing trails which will lead them to a happier, more healthful existence. With ample food supplies they will be better able to organize, and cope with the problems of defending what they must grow and of leading a peaceful existence with the rest of the world.

Disease Agents Recovered Incidental to a Tick Survey of the Mississippi Gulf Coast[1]

CORNELIUS B. PHILIP[2] and JESSE S. WHITE[3]

Ixodid ticks collected by the junior author (White 1949) incidental to a survey in Jackson County, Mississippi, were forwarded to the Rocky Mountain Laboratory, Hamilton, Montana, for examination for disease agents. Jackson County is the most eastward of the three coastal counties of Mississippi. In all, five species of Ixodidae were collected between 25 August 1947 and 28 May 1949, but disease agents were recovered in only the two most prevalent species in collections, namely, *Amblyomma americanum* (L.) and *A. maculatum* Koch. The results of these tick tests are the subject of this report.[4]

MATERIALS AND METHODS.—The main source of ticks was domestic animals, supplemented by smaller numbers of unfed ticks taken by dragging vegetation with a flannel cloth. The ticks were numbered according to a given collection and date off one host species or from one location, and air mailed alive under permit to the Laboratory. They were then sorted by species into groups of not over 25 for test. Each group of fed ticks off animals was tested by direct intraperitoneal injection into duplicate, male guinea pigs and unfed, "drag ticks" were given a preliminary feeding of 6 days on guinea pigs before similar injection into other test animals.

Tick suspensions were prepared by an initial immersion of the whole ticks for 1 hour in 1:1000 merthiolate solution to reduce surface contaminants, followed by vigorous washing in several changes of sterile, distilled water, and by subsequent trituration in appropriate amounts of physiological salt solution, normally not over 8 ml. to each group of 25 ticks. The resulting suspension, minus the heavy sediment, was divided for injection into two male, test animals.

Temperatures were recorded for 20 days, or in the event of fever and/or scrotal swelling, the animal was sacrificed usually on the second day of fever. Transfers of testicular washings, plus tunica or spleen tissue in some instances, were made to two fresh, male guinea pigs. Each suspected isolate was maintained in series by similar transfer until identified. Some animals in each series which remained afebrile or which recovered from apparent infection, were bled by heart puncture under anesthesia on or shortly after the 20th day for serological tests.

Each was then challenged with appropriate, known rickettsial agents to test for immunity. The strains used were the virulent Sheila Smith Rocky Mountain spotted fever, and a previous laboratory isolate of "maculatum" agent from Texas, plus, in most instances, another later challenge with "Nine-mile" strain of Q fever. In a few instances as indicated, immunity to endemic typhus was tested using the Wilmington strain of *Rickettsia typhi*. Criteria for identification of the "maculatum" agent also included challenge of spotted fever-vaccinated guinea pigs with the suspected tick-isolate, since the vaccine affords a low degree of protection against this agent. Simultaneous protection of animals by this vaccine was shown against a virulent, passage strain of spotted fever.

Complement-fixation tests on guinea pig sera and a few from local residents were accomplished in the unit of Dr. D. B. Lackman. Mr. G. M. Kohls identified and sorted the tick lots when received.

EXPERIMENTAL OBSERVATIONS.—Two rickettsial disease agents were recovered in tests of ticks from the Mississippi Gulf Coast. In all, 35 lots of ticks, mostly adults, were received for test. These comprised the following species: *Amblyomma maculatum*, 1806 from sheep, 6 from cattle, and 18 from dogs; *A. americanum*, 617 from sheep, 88 from cattle, 417 from dogs, 54 from pigs, 2 from goats, and 37 drag ticks; *Dermacentor variabilis* (Say), 16 from dogs and 2 from sheep; *Ixodes scapularis* Say, 99 from sheep and 74 from goats; and *I. cookei* Pack., 20 from dogs.

Though much work and many guinea pigs are involved in tests producing negative or rarely doubtful results, only the protocols for the tests of tick lots providing verified isolates of two rickettsial agents are discussed here.

Coxiella burnetii.—The agent of Q fever was isolated in tests of 31 adults and 7 nymphs, Lot 5, of the lone star tick, *A. americanum*, which were collected off vegetation on 8 May 1948, near Ocean Springs, Mississippi.

The ticks were divided into two groups for feeding: 25 adults attached to guinea pig C9652, and 6 adults and 7 nymphs to another guinea pig, C9653. Six days later both groups were removed and respective suspensions were injected in duplicate into guinea pigs C9846–7, and C9848–9. Transfers from the fourth animal were maintained for three more guinea pig passages, as diagramed in chart 1, while the isolate was being identified.

Both of the original guinea pigs on which the ticks fed, died of intercurrent infection on the seventh and eighth days without temperature rise or lesions suggestive of Q fever. Injection of tick suspension from group one resulted negatively. From group 2, one animal had 8 days of fever beginning on the 17th day, but died on the 41st day of apparent pneumonia. However, serum of the 29th day gave positive complement fixation in dilution of 1:2048. The other was sacrificed on the third day of fever after a 3-day incubation period. The spleen was slightly enlarged on necropsy and there were hemorrhagic areas in the lungs. A pool of tunica and spleen was transferred to four guinea pigs. One of these had 5 days of low fever after incubation of 1 week, and was immune to challenge with "Nine-mile" passage strain virus on the 33rd day. Serum drawn on the 31st day had complement-fixing antibodies against *C. burnetii*. The second developed 7 days of fever after 1 week and was sacrificed for

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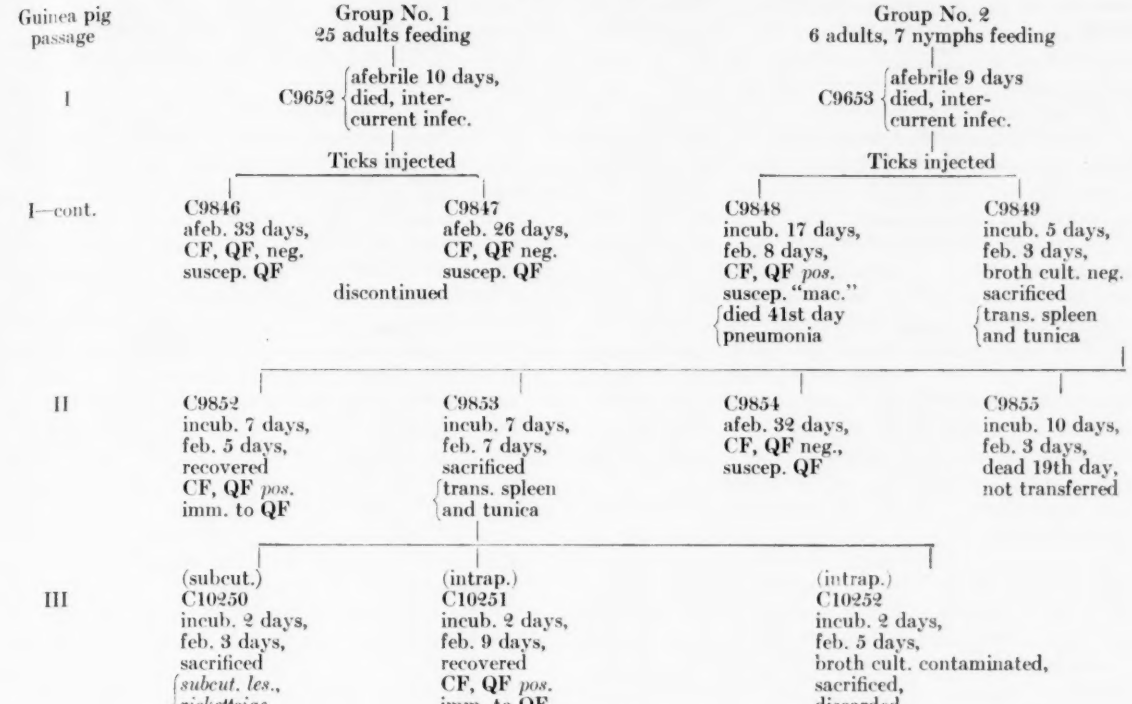
[2] From the U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Montana.

[3] From the Delta State Teachers College, Cleveland, Mississippi.

[4] The laboratory tests were performed under the direction of the late Dr. R. R. Parker.

CHART 1.—Isolation and identification in guinea pigs of *Coxiella burnetii* from *Amblyomma americanum*, Lot 5.


Guinea pig passage
Group No. 1
25 adults feeding
Group No. 2
6 adults, 7 nymphs feeding
I
C9652 afebrile 10 days, died, intercurrent infec.
C9653 afebrile 9 days died, intercurrent infec.
Ticks injected
Ticks injected
I—cont.
C9846 afeb. 33 days, CF, QF, neg. suscep. QF
C9847 afeb. 26 days, CF, QF neg. suscep. QF discontinued
C9848 incub. 17 days, feb. 8 days, CF, QF pos. suscep. "mac." died 41st day pneumonia
C9849 incub. 5 days, feb. 3 days, broth cult. neg. sacrificed trans. spleen and tunica
II
C9852 incub. 7 days, feb. 5 days, recovered CF, QF pos. imm. to QF
C9853 incub. 7 days, feb. 7 days, sacrificed trans. spleen and tunica
C9854 afeb. 32 days, CF, QF neg., suscep. QF
C9855 incub. 10 days, feb. 3 days, dead 19th day, not transferred
III
(subcut.) C10250 incub. 2 days, feb. 3 days, sacrificed subcut. les., rickettsiae in smear broth cult.—neg. passed to IV
(intrap.) C10251 incub. 2 days, feb. 9 days, recovered CF, QF pos. imm. to QF, suscep. "mac." agent
(intrap.) C10252 incub. 2 days, feb. 5 days, broth cult. contaminated, sacrificed, discarded

transfer by pool of spleen and tunica to the third passage. The third animal was afebrile for 36 days and its susceptibility to challenge indicated that this animal did not receive an infectious dose. The fourth animal had 3 days of low fever beginning on the tenth day and died on the 19th with suggestive lesions and clear lungs, but it was not transferred.

One of three guinea pigs of the third passage from the second animal above developed a characteristic skin lesion at site of subcutaneous injection after 2 days' incubation and 3 days of fever; it was used for subcutaneous transfer to the fourth passage. The second animal had 9 days of fever after a 2-day incubation period, and on recovery was immune to challenge with the "Nine-mile" passage virus. Serum on the 29th day, before challenge, also showed complement-fixing antibodies against antigens of both the Henzerling and "Nine-mile" strains. The third animal became febrile, but heart blood culture in broth yielded a Gram-negative bacillus and the animal was discarded.

Both transfer animals of the fourth passage developed characteristic febrile disease and subcutaneous lesions. Rickettsiae were found on Giemsa-stained imprints from the skin lesion. Heart blood culture in broth from each showed no contaminative growth.

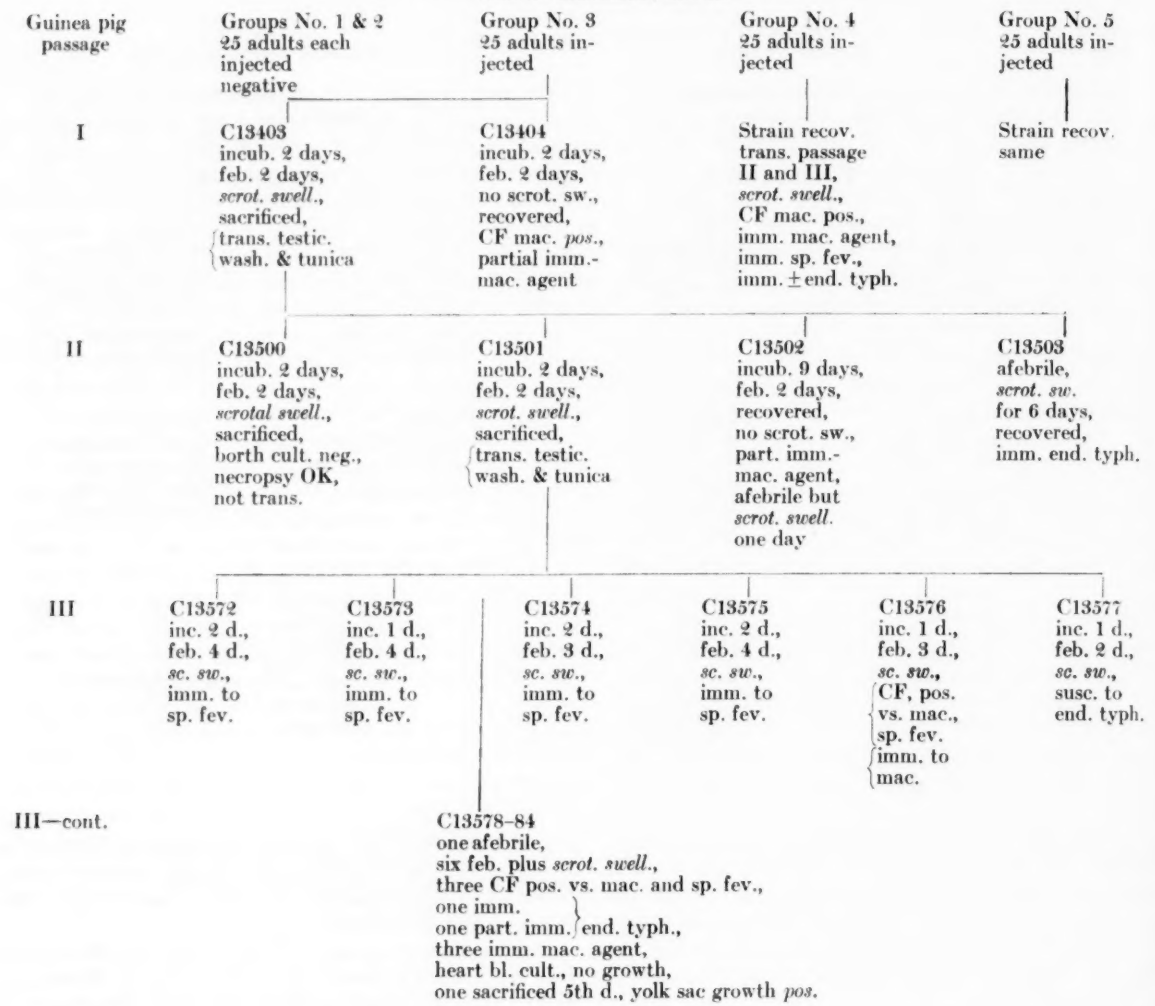
It is apparent that a strain of Q fever rickettsiae was isolated from this lot of ticks. Spontaneous infection of test guinea pigs, which has been encountered in an occasional experimental animal in other studies, can hardly account for evidence of infection in *both* the original tick-injected animals. The positive complement fixation in serum of one, and isolation of a strain from the other, leave little doubt that the original *A. americanum* were infected with *C. burnetii*.

The "maculatum agent."—Eight isolates of this rickettsial agent were recovered from four lots of Mississippi ticks. A sample isolation from one lot is diagramed in chart 2.

Lot 8. From sheep near Ocean Springs, 488 *A. maculatum* were received of which only 80 survived transportation. The live ticks were divided into 2 groups of 25 and 3 of 10 for injection into guinea pigs. Only group 4 consisting of a suspension of 10 fed female ticks caused reaction. Both guinea pigs developed 2 days of low fever beginning on the third day, plus scrotal swelling. One was immune to challenge on the 27th day with controlled "maculatum virus," and was susceptible on the 40th day to challenge with "Nine-mile" Q fever. The other was sacrificed on the second day of fever for passage. At necropsy the lungs were clear, the spleen was not enlarged, the testes and tunica were inflamed and had slight exudate, and there was no growth in broth culture of heart blood.

For transfer, the testes and tunica were agitated in a flask with glass beads and 8 ml. of sterile physiological salt solution, and 2 ml. of the washings were injected intraperitoneally into each of four guinea pigs. Each of these first transfer animals developed fever in 2 to 3 days, and scrotal reaction. Two were sacrificed on the second day of fever, and both showed the same findings at

CHART 2.—Isolation and identification in guinea pigs of "maculatum disease" agent from *A. maculatum*, Lot 25.


Guinea pig passage
Groups No. 1 & 2 25 adults each injected negative
Group No. 3 25 adults injected
Group No. 4 25 adults injected
Group No. 5 25 adults injected
I
C13403 incub. 2 days, feb. 2 days, *scrot. swell.*, sacrificed, trans. testic. wash. & tunica
C13404 incub. 2 days, feb. 2 days, no scrot. sw., recovered, CF mac. *pos.*, partial imm.-mac. agent
Strain recov. trans. passage II and III, *scrot. swell.*, CF mac. pos., imm. mac. agent, imm. sp. fev., imm. ± end. typh.
Strain recov. same
II
C13500 incub. 2 days, feb. 2 days, *scrotal swell.*, sacrificed, borth cult. neg., necropsy OK, not trans.
C13501 incub. 2 days, feb. 2 days, *scrot. swell.*, sacrificed, trans. testic. wash. & tunica
C13502 incub. 9 days, feb. 2 days, recovered, no scrot. sw., part. imm.-mac. agent, afebrile but *scrot. swell.* one day
C13503 afebrile, *scrot. sw.* for 6 days, recovered, imm. end. typh.
III
C13572 inc. 2 d., feb. 4 d., *sc. sw.*, imm. to sp. fev.
C13573 inc. 1 d., feb. 4 d., *sc. sw.*, imm. to sp. fev.
C13574 inc. 2 d., feb. 3 d., *sc. sw.*, imm. to sp. fev.
C13575 inc. 2 d., feb. 4 d., *sc. sw.*, imm. to sp. fev.
C13576 inc. 1 d., feb. 3 d., *sc. sw.*, CF, pos. vs. mac., sp. fev. imm. to mac.
C13577 inc. 1 d., feb. 2 d., *sc. sw.*, susc. to end. typh.
III—cont.
C13578–84 one afebrile, six feb. plus *scrot. swell.*, three CF pos. vs. mac. and sp. fev., one imm. one part. imm. end. typh., three imm. mac. agent, heart bl. cult., no growth, one sacrificed 5th d., yolk sac growth *pos.*

necropsy as the original animal. Blood cultures were without growth. Testicular washings of one animal were transferred to six guinea pigs. The two remaining animals recovered and their serums on the 21st day provided complement-fixation tests against known "maculatum" antigen in dilutions of 1:16. In later challenges, neither showed reaction to controlled "maculatum virus" but were both susceptible to "Nine-mile" Q fever.

In this manner, the isolate was maintained for seven more passages involving 21 guinea pigs, all of which reacted similarly. Nine of these were sacrificed for further infection studies. Three others gave complement-fixation tests against "maculatum" antigen at about 3 weeks, and all 12 of the recovered animals were challenged with one or another of the following three laboratory passage agents. Four each were immune to "maculatum" and spotted fever strains; three others showed fever and scrotal swelling following injection with endemic typhus, Wilmington strain, but a fourth was resistant.

Lot 17. From sheep at Vancleave, 83 live and 41 dead adult *A. maculatum*, and 10 live adult *A. americanum* were received. Five groups of both live and dead ticks of the former, and a sixth of *A. americanum* were tested in two guinea pigs each. Four groups, including the *A. americanum*, caused no reaction, and the test animals were susceptible to primary and secondary challenges with "maculatum virus" and "Nine-mile" Q. fever. The test of one group by injection of a suspension of dead *A. maculatum* was invalidated by intercurrent infection. From group 1, consisting of a suspension of 25 live *A. maculatum* adults, a strain was established. One of the two test animals showed only one day of low fever and no scrotal reaction. The complement-fixation test of its serum on the 24th day was negative and it was susceptible to challenge with "maculatum virus."

The duplicate animal from group 1 developed 2 days of fever beginning on the second day and scrotal swelling when it was sacrificed for passage. Blood culture showed no growth. The strain was maintained for five passages by testicular washings, with results similar to tests of the

preceding lot. Four of the recovered animals had complement-fixing antibodies against "maculatum" antigen before challenge. Four recovered animals each were immune to challenge with "maculatum" and spotted fever strains. Of four others challenged with endemic typhus, only one was immune, two were partially immune showing some fever but no scrotal reaction, and one was susceptible showing both fever and orchitis.

Lot 25. One hundred twenty five live *A. maculatum* were received from sheep at Escatawpa and tested in five groups of 25 each, as diagramed in chart 2. No reaction occurred in tests of the first two, and the guinea pigs were susceptible to challenge with both "maculatum" and spotted fever strains. Groups 3, 4, and 5 each provided strains, one of which was maintained for three passages, and the others for two. Serums from some recovered animals in each line gave positive complement-fixation with antigens of both "maculatum" and spotted fever. Four recovered animals each from groups 3 and 4, and three each from group 5 were immune to challenge with either "maculatum" or spotted fever strains. From group 5, one was only partially immune to "maculatum virus," and another showed fever and scrotal swelling on challenge with spotted fever. Reactions were variable after endemic typhus challenge; in three groups, 3, 2, and 3 animals, respectively, were immune, and the remainder were partially immune showing low fevers but no scrotal involvement.

Lot 28. From other Escatawpa sheep, 247 *A. maculatum* adults were collected and forwarded; 200 live ticks were divided into eight groups, and 47 dead ones into two others. Three strains of "maculatum agent" were again isolated from groups 4, 7, and 10 (dead ticks). The test animals of the other 7 groups remained afebrile and were susceptible to both "maculatum virus" and spotted fever challenge.

Each of the three isolates was maintained for two passages, and positive complement-fixation reactions were obtained in two of the lines before challenge of recoveries. Four recovered animals each in passages of groups 4 and 7, and three each in group 10 were immune to "maculatum" and spotted fever challenge. One each of group 10 had doubtful immunity to these two strains. All four recovered animals that were challenged with endemic typhus in group 10, and three in group 4, were immune; the fourth in the latter was only partially immune showing brief low fever but no scrotal involvement. All four in group 7 were susceptible to endemic typhus and showed both fever and orchitis.

VACCINATION TESTS.—A total of 40 guinea pigs, including controls, was injected with 1 ml. of tick-type Rocky Mountain spotted fever vaccine that gave full protection against virulent Sheila Smith strain on challenge. Temperatures were taken for 10 days after vaccination and any showing unexplained fevers were discarded. Isolates from all the above four lots of ticks were then used to challenge these vaccinated animals in groups on the 11th day. All but one of the animals showed fever and scrotal involvement after challenge with respective sublot strains as follows: Lot 8 sublot 4, 4 guinea pigs; Lot 17-1, 4; Lot 25-3, -4, and -5, 4 guinea pigs each; Lot 28-4 and -7, 4 each; Lot 28-10, 3 guinea pigs. The only test animal that completely resisted challenge and remained afebrile was in the last sublot, 28-10; six vaccinated control animals were fully protected against spotted fever.

DISCUSSION.—This is the first time that the Q fever agent, *C. burnetii*, has been found in ticks in Mississippi. Since ticks naturally infected with this human pathogen are now known to be widespread in North America, and transmission by tick bite to experimental laboratory animals is readily demonstrated, it is increasingly puzzling why human cases following tick bite are not reported. Eklund *et al.* (1947) and McGurl & Williams (1948) have recorded the only possible cases with history of tick attachment to date in this country. Doubt is even increasing that tick feces-contaminated hides are as responsible in abattoir infections in Australia as originally surmised. Nevertheless, ticks can furnish an important link in the chain of natural maintenance.

The "maculatum disease" agent was first described by Parker *et al.* (1939) from *A. maculatum* taken off cows in Texas. They showed that this agent was immunologically closer to spotted fever than to endemic typhus in experimental animal studies, and that it consistently caused milder disease in guinea pigs than the usual laboratory strains of spotted fever. Lackman *et al.* (1949) made a serological study of "maculatum" strains recovered from ticks in Texas, Georgia, and Mississippi compared with other rickettsial agents, and confirmed that the former belonged in the spotted fever group but were not identical to any of those related agents. Homologous complement-fixing titers were consistently and significantly higher than heterologous titers. As in fièvre boutonneuse and North Queensland "tick typhus," the "maculatum" agent elicits no *Proteus* agglutinin response in recovered rabbit serums, whereas spotted fever does.

The "maculatum" agent is not known to infect man, and so far as tested, it causes frank though mild disease only in guinea pigs. Antigens have been prepared from infected yolk sacs of embryonated chicken eggs.

Cases of Rocky Mountain spotted fever have been reported over nearly a decade from eight widely distributed counties in Mississippi including Jackson County where this tick survey was carried on. Two cases occurred near Biloxi in 1948, both of which had complement-fixing antibodies and showed rising titers of *Proteus* OX_{19} agglutinins in serially-drawn serum samples. Since *A. americanum* is a known vector of *R. rickettsii*, it is surprising that strains were not recovered in the considerable number of this species tested from Jackson County. Serums of two other residents of the county also provided serological evidence of past experience with this disease. Two of these uninfected lots of *A. americanum* were from a dog and two pigs on the Biloxi farm where one of the proved cases in 1948 occurred.

SUMMARY.—During an ixodid tick survey extending over nearly 2½ years in Jackson County, Mississippi, one strain of Q fever and eight of "maculatum disease" agent were recovered in tests of *Amblyomma americanum* and *A. maculatum* taken off domestic livestock or vegetation. Two cases of Rocky Mountain spotted fever occurred in the county during the survey but no strains of this disease agent were recovered from local ticks.

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Ixodid Ticks from the Mississippi Gulf Coast[1]

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A survey of ixodid ticks in Jackson County, the most eastward of the three coastal counties of Mississippi, between August 25, 1947, and May 28, 1949, revealed the presence of six species. Identifications based on field collections showed the tick population on domesticated animals to be composed for four genera and five species as follows: *Amblyomma americanum* (L.,) the lone star tick; *A. maculatum* Koch, the Gulf Coast tick; *Dermacentor variabilis* (Say), the American dog tick; *Ixodes scapularis* Say, the black-legged tick; and, *Rhipicephalus sanguineus* (Latr.), the brown dog tick. The rabbit tick, *Haemaphysalis leporis-palustris* (Pack.), was collected from specimens of *Sylvilagus floridanus alacer*, cottontail rabbit.

The two species of *Amblyomma* were examined at the Rocky Mountain Laboratory for the presence of disease agents (see following report by Philip & White). Live ticks were forwarded by the author for these infectivity experiments.

METHODS AND MATERIALS.—Collections were made from domesticated animals encountered in a house to house and farm to farm canvas. Cows, sheep, and hogs in the barnyards, and dogs, were routinely examined. Sheep proved to be the main hosts of *A. maculatum* adults; thus considerable time was expended in locating, and after locating, in interesting the owners of herds to pen the animals in small enclosures. Arrangements were made several days in advance for large herds of sheep to be brought in from the open range. These animals were usually herded into the small pens adjacent to a barn typically used at shearing time. The co-operativeness of owners of livestock helped to further the work of this survey.

Since only live ticks were usable in the infectivity experiments, the exercise of care in removal of the specimens with forceps from their point of attachment was one means whereby survival in shipment could be increased. Injury was likely the cause of a fatality rate which exceeded 25% in some shipments. Upon removal in the field, the ticks were collected in pint bottles containing blades of grass. The ticks were transferred usually within a period of 1 to 6 hours and sorted in pill boxes for packing and shipment. Over-crowding was avoided. Inclusion of chopped blades of grass to increase humidity facilitated survival of the ticks in transit. These collections, plus data, were forwarded air mail in heavy cardboard, screw-top mailing tubes with shipping permits issued by the former Bureau of Entomology and Plant Quarantine. Shipments from Ocean Springs, Mississippi, to Hamilton, Montana, by air required 72 to 96 hours.

OBSERVATIONS.—Shipments were initiated on March 27, 1948, and continued through May 28, 1949. Table 1 lists the collection data on which these shipments were based and also indicates in which laboratory tests *A. americanum* and *A. maculatum* were found to be positive for disease agents.

ATTACHMENT AND HOST LIST.—On several occasions inhabitants of the county described the nuisance of heavy tick infestation in the grass of lawns and on shrubbery, and they often found ticks attached to their persons after working in the yard. Many specimens were collected from this source, comprising mainly larvae, nymphs, and adults of *Amblyomma americanum*, but including also *Ixodes scapularis* especially in the winter months from November through February. Collections of three species of ticks were facilitated by the use of a white flannel drag.

Immature stages of *A. maculatum* were not found attached to man in Jackson County, and only four cases of complete attachment of adults to man can be reported in this survey. Thirty minutes to an hour following the deticking of a herd of 30 sheep, the author found one adult male attached on the underside of the left fore arm.

In general, *A. maculatum* on sheep prefers to attach in clusters, shingle style, inside the middle or distal third of the external ear; however, they will attach outside the ear on the distal third to the tip. Only rarely was it found attached to any other part of the body, but individual specimens were found on the lower eyelid, fore cheek, and hind cheek, and around the base of the horns. On sheep the oily areas are avoided; thus ticks were not found in the wool and on the medial surfaces of the hind legs and flanks.

Hixson (1940) discussed the conditions created in the

[1] Field studies were carried on in cooperation with the Jackson County Health Department, Dr. Andrew Hedmeg, then Director; the Mississippi State Board of Health, Dr. A. L. Gray, Director of CDC Activities; The Rocky Mountain Laboratory, Public Health Service, the late Dr. R. R. Parker, then Director; and, The Gulf Coast Research Laboratory Project of the Mississippi Academy of Sciences, Inc., Dr. R. L. Caylor, then Director, with acknowledgments to Dr. Emil Kotcher and Dr. J. W. Ward. This report contains data from a thesis prepared with the guidance of Dr. E. W. Stafford and submitted in partial fulfillment of the requirements for the degree of Master of Science at Mississippi State College, State College, August 1949. Accepted for publication January 19, 1955.

Table 1.—Collection data on shipments of living *Amblyomma maculatum* and *A. americanum* and results of laboratory tests of these ticks for disease agents.

Tick Lot No.	Date Collected	Locality	Host or Source	Species and Number of Ticks	Laboratory Report*
1	3-28-48	Ocean Springs	1 dog	*americanum*, 355 adults	Negative
2	3-27-48	Ocean Springs	1 dog	*americanum*, 63 adults	Negative
3	3-27-48	Ocean Springs	1 cow	*americanum*, 38 adults	Negative
4	5- 8-48	Ocean Springs	56 sheep	*maculatum*, 18 adults	Negative
5	5- 8-48	Ocean Springs	vegetation	*americanum*, 31 adults 7 nymphs	Positive
8	7-24-48	Ocean Springs	100 sheep	*maculatum*, 488 adults	Positive
9	7-24-48	Ocean Springs	12 sheep	*maculatum*, 20 adults	Negative
10	7-24-48	Ocean Springs	100 sheep	*americanum*, 24 adults	Negative
11	7-29-48	Latimer Community	1 dog	*americanum*, 7 adults *maculatum*, 2 adults	Negative Negative
12	7-29-48	Latimer Community	2 pigs	*americanum*, 5 adults 16 nymphs	Negative
	7-29-48	Latimer Community	1 calf	*maculatum*, 1 adult	Not tested
13	8- 3-48	Latimer Community	4 cows	*americanum*, 13 adults 8 nymphs	Negative
14	8- 5-48	Ocean Springs	9 sheep	*americanum*, 15 adults 11 nymphs	Negative
				maculatum, 42 adults	Negative
15	8- 7-48	Vancleave	12 sheep	*maculatum*, 77 adults *americanum*, 12 adults	Negative Negative
16	8- 7-48	Ocean Springs	19 sheep	*maculatum*, 154 adults *americanum*, 21 adults	Negative Negative
17	8- 9-48	Vancleave	24 sheep	*maculatum*, 128 adults *americanum*, 10 adults	Positive Negative
18	8- 9-48	Vancleave	5 sheep	*maculatum*, 9 adults	Negative
19	8- 9-48	Vancleave	1 dog	*maculatum*, 10 adults *americanum*, 31 adults	Negative Negative
20	8-10-48	Ocean Springs	42 sheep	*maculatum*, 273 adults *americanum*, 73 adults	Negative Negative
21	8-18-48	Ocean Springs	2 dogs	*maculatum*, 7 adults	Negative
22	8-18-48	Latimer Community	3 pigs	*americanum*, 33 nymphs	Negative
23	8-19-48	Gautier	104 sheep	*maculatum*, 37 adults *americanum*, 175 adults, 77 nymphs	Negative Negative
24	8-24-48	Ocean Springs	42 sheep	*maculatum*, 245 adults *americanum*, 152 adults, 20 nymphs	Negative Negative
25	8-25-48	Escatawpa	20 sheep	*maculatum*, 126 adults	Positive
26	8-27-48	Big Point	25 sheep	*maculatum*, 45 adults	Negative
27	8-31-48	Hurley	70 sheep	*maculatum*, 104 adults	Negative
28	9- 1-48	Escatawpa	72 sheep	*maculatum*, 246 adults	Positive
28A	8-28-48	From eggs of females of A. P. 25108		*maculatum*, 100 plus, 2 month old larvae	Negative
29	10-13-48	Escatawpa	45 sheep	*maculatum*, 28 adults	Negative
30	10-13-48	Gautier	50 sheep	*maculatum*, 4 adults *americanum*, 4 adults	Negative Negative
32	10-14-48	Ocean Springs	30 goats	*maculatum*, 2 adults	Negative
36	5-28-49	Ocean Springs	26 sheep	*maculatum*, 12 adults *americnaum*, 11 adults, 1 nymph	Negative Negative
35	5-28-49	Ocean Springs	12 sheep	*americanum*, 13 adults	Negative
34	5-28-49	Ocean Springs	3 dogs	*americanum*, 7 adults	Negative
33	5-28-49	Ocean Springs	5 sheep	*americanum*, 1 adult *maculatum*, 1 adult	Negative Negative

* See Philip and White (1955) for protocols.

ears of domestic animals by *A. maculatum* with reference to screw worm infestations in Georgia. Infestations of screw worms were not observed in the ears of sheep during this survey of Jackson County, Mississippi; however, the ears of the majority of sheep parasitized by ticks were in poor condition. On one occasion thirty adult ticks were removed from the badly swollen and drooped ears of one ewe.

Amblyomma maculatum was found attacked inside the middle or distal third of the ear of dogs, and frequently in the long hair mid-dorsally in the cervical and thoracic regions. The place of attachment seems to be of far less consequence to *A. americanum*. It was observed to attach to almost any part of the body of cows, dogs, horses, and hogs. On sheep it is limited to nonoily areas, the ears being the most frequent area of attachment.

On a collecting trip on November 12 to 14, 1948, *Ixodes scapularis* was found to be the most abundant species on sheep, goats, dogs, and cows. The decrease in numbers of *A. maculatum* in the late fall was readily noticeable and *A. americanum* was scarce. *I. scapularis* attached mainly at the outer base of the ear, rarely inside the ear. It was also found in the whiskers under the chin of goats, along the underside of the neck of goats and cows, and under the throat of sheep.

The hosts from which ticks[2] were obtained between August 25, 1947, and May 28, 1949, are given in the accompanying list. The following are included: man, domesticated animals, birds, one reptile; also, from vegetation by drag.

Amblyomma americanum

Adults: Man; cats; cows; dogs; horses; hogs; sheep; Gray fox, *Urocyon cinereoargenteus* subsp.
From vegetation by white flannel drag.

Nymphs: Man, cats, cows, dogs, horses, hogs.
Meadowlark, *Sturnella magna;* Towhee, *Pipilo erythrophthalmus;* Brown Thrasher, *Toxostoma rufum;* Bob-white, *Colinus virginianus.*
From vegetation by white flannel drag.

Larvae: Man; cows; hogs; dogs; Cottontail rabbit, *Sylvilagus floridanus alacer.*
Meadowlark, *Sturnella magna;* Towhee, *Pipilo erythrophthalmus;* Wood Thrush, *Hylocichla mustelina.*
From vegetation by white flannel drag.

Based upon 3564 ticks, this species represented approximately 52% of the total collections.

Amblyomma maculatum

Adults: Man, cows, dogs, horses, hogs, sheep.
From vegetation by clothing.

Nymphs: Meadowlark, *Sturnella magna.*

Larvae: Meadowlark, *Sturnella magna;* Cardinal, *Richmondena cardinalis.*

Based upon 2781 ticks, this species represented approximately 40% of the total collections.

Dermacentor variabilis

Adults: Dogs; also, cow and sheep in one instance each.
One specimen from man.

Based upon 60 ticks, it represented less than 1% of the total collections.

Haemaphysalis leporis-palustris

Adults and nymphs: Cottontail rabbit. *Sylvilagus floridanus alacer.*

Larvae: One larva of *Haemaphysalis* sp. from a Cardinal, *Richmondena cardinalis.*

Based on 102 ticks, it represented above 1% of the total collections.

Ixodes scapularis

Adults: Man; cows; dogs; goats; horses; hogs; sheep; Gray fox, *Urocyon cinereoargenteus* subsp.
From vegetation by white flannel drag.

Nymphs: One from a cow.
One engorged nymph was found attached immediately posterior to the left fore leg of a skink, *Eumeces fasciatus.*

Based on 346 ticks, it represented approximately 5% of the total collections.

Rhipicephalus sanguineus

Adults: Only one dog was found infested.

Based on 4 ticks, it represented less than 1% of the total collections.

SUMMARY.—A total of 6857 ixodid ticks was collected during a survey in Jackson County, Mississippi, between March 1948 and May 1949. These comprised five genera and six species as follows: *Amblyomma americanum, A. maculatum, Dermacentor variabilis, Ixodes scapularis, Rhipicephalus sanguineus,* collected from domesticated animals and *Haemaphysalis leporis-palustris* from cottontail rabbits.

Attacks on man by all stages of *A. americanum* are frequent but only four instances were observed in which *A. maculatum,* all adult ticks, were taken off persons.

The survey was successful in supplying live ticks in sufficient quantities to warrant laboratory experiments designed to test the ticks for disease agents. Approximately 1200 specimens of *A. americanum* and 2200 specimens of *A maculatum* were shipped by air mail to the Rocky Mountain Laboratory for such tests, the results of which are reported in the next paper.

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[2] The identifications of a series of immature ticks from birds and wild animals were made by Glen M. Kohls, Rocky Mountain Laboratory, Hamilton, Montana.

Mechanism of Action of Certain Synergists for DDT against Resistant House Flies[1]

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Certain compounds structurally related to DDT, [2,2-bis (*p*-chlorophenyl)-1,1,1-trichloroethane], may act as synergists for DDT against insecticide resistant house flies (Summerford *et al.*, 1951 March *et al.* 1952). Three obvious questions come to mind with respect to these findings. First, how do these synergists exert their effect; second, by what mechanism can the house fly eventually become resistant to the combination (March *et al.* 1952); and third, why do these synergists potentiate the toxicity of DDT for resistant flies but not for susceptible flies? The answer to these questions would have an important bearing upon the significance of the fact that DDT resistant flies rapidly dehydrochlorinate DDT (Sternburg *et al.* 1950, March & Metcalf 1950, Winteringham *et al.* 1951). Perry *et al.* (1953) propose that the synergist DMC, [bis (*p*-chlorophenyl) methylcarbinol], acts by interfering with the mechanism which detoxifies DDT. Since these experiments were necessarily carried out by *in vivo* procedures, their proposal might be questioned as to whether this is actually the cause or merely the result of synergism.

Sternburg *et al.* (1953) successfully demonstrated the presence of an enzyme in DDT-resistant house flies which catalyzes the dehydrochlorination of DDT to its non-toxic derivative DDE,[2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene]. More recently Sternburg *et al.* (1954) have shown this enzyme, DDT-dehydrochlorinase, to be present in all DDT-resistant strains tested and have devised methods for its assay and partial purification. It therefore seemed logical to determine 1) if the so-called DDT synergists can inhibit DDT-dehydrochlorinase, 2) if DDT-dehydrochlorinase has any effect upon the synergists and, 3) if there is any determinable difference in DDT-dehydrochlorinase derived from flies resistant only to DDT and that derived from flies resistant to the combination of synergist and DDT.

MATERIALS AND METHODS.—The sources of enzyme used in these studies were acetone powders prepared from four different strains of flies. The comparative experiments, determining absolute levels of DDT-dehydrochlorinase in the various strains, were performed on equivalent homogenates. The homogenates consisted of 200 adult flies ground in 20 ml. of cold, distilled water. The "S strain", which has maintained a high level of resistance for the past 3 years, was frequently exposed to DDT in the standard larval fly medium. A high proportion of the adults in each generation of this strain was essentially immune to DDT. The "DMC strain" was produced through selection of DDT-resistant flies exposed for 15 minutes to space sprays containing 600 mg. DDT plus 300 mg. DMC in 12 ml. of spray solution. These solutions were applied to flies in a Peet-Grady chamber, and the survivors were inbred for each succeeding generation (Schoof 1954). The "Synergist strain," originally a DDT-resistant strain collected from the field, was further selected by exposing both larvae and adults to a pressure of 5:1 mixtures of DDT and bis (*p*-chlorophenyl) ethane permitting only 20% to 40% survival. All breeding stock was in turn individually treated within 24 hours after emergence with a mixture of 100 μgm. DDT plus 20 μgm. bis (*p*-chlorophenyl) chloromethane (March 1954).

The enzyme reactions were carried out in single sidearm Warburg vessels at 37° C. The substate and inhibitors were first crystallized from an acetone solution onto glass beads 15 to 60 microns in diameter, on the bottom of the flasks. One hundred and fifty mg. of beads were used for each mg. of substrate and inhibitors. After delivering the enzyme preparation, 2 ml. of homogenate, and 0.5 ml. of 0.137 molar phosphate buffer, pH 7.4, the vessels were simultaneously gassed with nitrogen for 15 minutes and equilibrated with the bath temperature. Zero time was marked by the introduction of 3 mg. of glutathione in 0.5 ml. buffer from the sidearm, and the reaction was permitted to run for 90 minutes. Quantitative analyses of the DDT-DDE mixtures were made by treating the reaction mixture with sulfuric acid, extracting with cyclohexane and measuring the absorbance of aliquots of the cyclohexane layer at 241 and 260 mμ with a Beckman DU spectrophotometer. Standard calculation methods for two component systems then yielded the amount of the substrate, DDT, which had been enzymatically converted to DDE. These procedures, as well as the preparation of acetone powders and fly homogenates have recently been described in detail (Sternburg *et al.* 1954). It was first determined that none of the chemicals used in this study (other than the substrate, DDT) could be altered in any manner detectable by a change in their ultraviolet absorption spectra, either by the enzyme DDT-dehydrochlorinase, or by other enzymes present under the test conditions of these assays. The inhibitors were then merely treated as interfering substances in the ultraviolet determinations, and by measuring the absorbance of interference controls, corrections could be applied to reflect only the absorbance of the DDT and DDE. One further alteration in the original procedure was necessary. When derivatives of diphenylcarbinol were assayed, sulfuric acid which alters these compounds was replaced with 3 ml. of hydrochloric acid, and the vessels were heated for 5 minutes in a boiling water bath. The flask contents were then quantitatively transferred to separatory funnels and extracted in the usual manner with cyclohexane.

A minimum of three replications was made on all data presented on the inhibitors, and each individual determination was performed in duplicate. Percentage inhibition was calculated as follows:

$$100-\left(\frac{\text{μgm. DDE produced in presence of inhibitor}}{\text{μgm. DDE produced in uninhibited control}}\times 100\right)=\%\ \text{Inhibition}$$

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Table 1.—Per cent inhibition of DDT-dehydrochlorinase by analogs of DDT.

Group	Compound	Mole Ratio Inhibitor to DDT[a]							Relative Synergistic Activity[b] (Activity of DDT Alone = 1)
		1:1	1:2	1:4	1:8	1:50	1:100	1:1000	
A	(1) 2,2-bis (*p*-chlorophenyl)-1,1-dichloroethylene (p-$ClC_6H_4)_2C=CCl_2$	0	0	0	0	0	0		2
	(2) 2,2-bis (phenyl)-1,1,1-trichloroethane ($C_6H_5)_2CHCCl_3$	0	0	0	0	0	0		1[c]
	(3) diphenylcarbinol ($C_6H_5)_2CHOH$	0	0	0	0				1
B	(4) 1,1-bis (*p*-chlorophenyl) ethanol[d] (p-$ClC_6H_4)_2CHCH_2OH$	25	15	0	0	0	0		30[e]
	(5) 1,1-bis (*p*-chlorophenyl) ethane (p-$ClC_6H_4)_2CHCH_3$	30	22	8	0	0	0		100
	(6) bis (*p*-chlorophenyl) methane (p-$ClC_6H_4)_2CH_2$	25	15	8	0				20
C	(7) bis (*p*-chlorophenyl) methylcarbinol (p-$ClC_6H_4)_2COCH_3$	70	63	63	68	66	64	18	60
	(8) bis (*p*-chlorophenyl) carbinol (p-$ClC_6H_4)_2CHOH$	64	62	64	67	59	52	12	3
	(9) bis (*p*-chlorophenyl) chloromethane[e] (p-$ClC_6H_4)_2CHCl$	65	60	60	67	66	59	10	140

[a] Substrate DDT maintained at a constant level of 4 mg.
[b] Determined by March *et al.* (1952) by topical application of microdrops.
[c] Determined by same procedure used by March *et al.* (1952).
[d] Supplied by Dr. F. A. Gunther, Citrus Expt. Station, Riverside, Calif.
[e] Supplied by Rohm and Haas Co., Philadelphia, Pa.

In order to test the reversibility of DDT-dehydrochlorinase after exposure to the various inhibitors, 15 ml. of an enzyme preparation was buffered with 5 ml. of phosphate, 25 mg. glutathione added and the whole introduced into a 125 ml. flask containing 10 mg. of the inhibitor crystallized on 500 mg. of glass beads. The mixture was shaken under nitrogen at 37° C. for 30 minutes, filtered through a double thickness of Whatman #1 filter paper and then centrifuged for 30 minutes at high speed (20,000×G) in an International (model PR-2) refrigerated centrifuge at 3° C. The upper portion of the supernatant liquid was carefully pipetted off and assayed on the standard substrate preparation of 4 mg. DDT. By comparing the activity of this enzyme solution with that of control enzyme solutions treated in the same manner but exposed to untreated beads, the degree of inhibition could be readily calculated. The degree of inhibition reversed by removal of the enzyme from contact with the synergist could be obtained by comparison with preparations held in constant contact with synergist and substrate.

Determinations of solubility of the inhibitors were made by evaporating 10 mg. of these compounds from acetone solution onto 500 mg. glass beads in 125 ml. flasks, adding 10 ml. deionized water and vigorously agitating on a wrist action shaker for 2 hours. The solutions were then filtered through double thickness of Whatman #50 filter paper. The filtrates were centrifuged at high speed for 30 minutes. Five ml. of the supernatant liquids were extracted with an equal quantity of cyclohexane, the absorbance of each read at 235 mμ, and the readings converted on standard calibration curves plotting known amounts of the compounds versus optical density at the same wavelength.

Discussion of Results.—The inhibitory effects of certain compounds structurally related to DDT, upon DDT-dehydrochlorinase are given in table 1. These data confirm the proposal of Perry *et al.* (1953) that DMC may act as a synergist by inhibiting the detoxication mechanism in DDT-resistant flies. It would have been desirable to have included all compounds reported to have a synergistic effect for DDT against DDT-resistant house flies. However, many of these compounds interfere with the methods of extraction and analysis presently used to measure enzymatic dehydrochlorination of DDT and therefore could not be tested. It would also have been desirable to have used the synergists in proportions greater than a 1:1 molar ratio with DDT. To do so would have necessitated the addition of increasing quantities of beads to the Warburg flask in order not to reduce the reaction surface of DDT below the minimum required for the maximum rate of enzymatic attack. The addition of greater quantities of beads interferes with extraction of the mixture and presents problems bearing on reaction rates which cannot be readily solved. In spite of these limitations, these data do provide answers to the questions previously raised.

Among the compounds listed in table 1, all those rec-

ognized as DDT-synergists (compounds 5, 6, 7 and 9) have the property of inhibiting DDT-dehydrochlorinase. On the other hand, one of the more effective inhibitors of DDT-dehydrochlorinase, bis (*p*-chlorophenyl) carbinol (8) was reported by March *et al.* (1952) to be only slightly effective as a DDT synergist. The cause for the ineffectiveness of this compound *in vivo* is not known, but it is apparent that one cannot conclude that compounds which inhibit DDT-dehydrochlorinase *in vitro* will do likewise *in vivo* even though the reverse may be true.

The ability of various compounds to synergize DDT against resistant flies does not quantitatively correlate with their ability to inhibit DDT-dehydrochlorinase *in vitro*. For example, March *et al.* (1952) found bis (*p*-chlorophenyl) methylcarbinol (7) to be only about one-half as effective as 1,1-bis (*p*-chlorophenyl) ethane (5) whereas, the opposite is true with respect to inhibition of DDT-dehydrochlorinase *in vitro*.

It is important to recognize the fact that a compound applied in combination with DDT to a house fly is subject to a variety of factors not encountered when the two are tested against DDT-dehydrochlorinase *in vitro*. The absorption, distribution and the ability of the fly to metabolize these compounds, together with still other side effects may in one way or another diminish the ability of the synergist to inhibit DDT-dehydrochlorinase *in vivo*. For the same reason one should not expect a quantitative correlation between inhibition of DDT-dehydrochlorinase *in vitro* and the synergistic effect of a compound against DDT-resistance in flies. In view of the prevailing evidence it seems more likely that a compound which exhibits synergistic effects with DDT in the intact fly will also inhibit DDT-dehydrochlorinase *in vitro*.

The compounds tested as inhibitors of DDT-dehydrochlorinase may be divided into three categories with respect to their effects; those which have no effect (Table 1, group A), another class capable of producing a moderate amount of inhibition (group B), and those which produce a marked and constant effect through a wide range of concentrations (group C).

The compounds listed in group B (Table 1) may be competitive inhibitors of DDT-dehydrochlorinase. Proof of this point would require a study of the kinetics of the reactions involving these inhibitors and DDT. Such a study is complicated by the two phase system represented in the reaction mixtures and by difficulties of extraction and analysis. Evidence in favor of this being the possible mechanism of inhibition is based upon the fact that the compounds in this group are structurally analogous to DDT and that their inhibitory effect on the enzyme may be largely reversed when the compound is removed from contact with the enzyme (Table 2).

The compounds listed in group C (Table 1), aside from being the most potent inhibitors tested, have certain properties in common which differentiate them from those in group B. They have, for example, a greater degree of solubility in water (Table 2) than those compounds listed in group B, and more important, the inhibition produced by these compounds at the concentrations tested cannot be reversed by removal of the insoluble portion of these compounds from contact with the enzyme (Table 2). It is reasonable to expect that a soluble inhibitor would be in a more favorable position to compete for active sites on a water soluble enzyme than the substrate which is present in a particulate state. However, solubility may not be a very important factor in the role of enzyme inhibition because diphenylcarbinol (3) is completely inactive as an inhibitor, whereas bis (*p*-chlorophenyl) carbinol (8) is a potent inhibitor and considerably less soluble in water than the former material.

Table 2.—Reversibility of Inhibition of DDT-Dehydrochlorinase.

Inhibitor[a]	μgm. DDE Produced in 90 Min.	Per Cent Inhibition	Per Cent Reversal[b]	Solubility in H_2O (p.p.m.)
Control (no previous exposure)	355	—	—	—
(1) 2,2-bis (*p*-chlorophenyl)-1,1-dichloroethylene	360	0	0	<1
(3) diphenylcarbinol	355	0	0	500
(4) 1,1-bis (*p*-chlorophenyl) ethanol	340	4	84	5
(5) 1,1-bis (*p*-chlorophenyl) ethane	330	7	76	<1
(7) bis (*p*-chlorophenyl) methylcarbinol	105	70	0	12
(8) bis (*p*-chlorophenyl) carbinol	136	63	2	9
(9) bis (*p*-chlorophenyl) chloromethane	140	61	6	10

[a] Procedure: Enzyme in contact with compound for 30 minutes under optimal conditions. Solution centrifuged, filtered and enzymatic activity assayed.
[b] Per cent reversal calculated by comparing inhibition produced by synergist in constant contact with enzyme at 1:1 mole ratio with DDT (Table 1) and that after removal of enzyme from synergist.

These findings tend to emphasize the importance of *p*-chloro substituents as one of the major factors determining the inhibitory effect of non-toxic DDT analogues on DDT-dehydrochlorinase and their performance as DDT synergists. It was earlier shown (Sternburg *et al.* 1954) that the para substituents on DDT were essential for enzymatic attack by DDT-dehydrochlorinase. This finding is substantiated by the fact that 2,2-bis (phenyl)-1,1,1-trichlorethane neither dehydrochlorinates nor competes with DDT for the enzyme sites in mixtures up to equimolar proportions. Diphenylcarbinol (Table 1), will not inhibit DDT-dehydrochlorinase and is useless as a synergist, but the same compound with *p*-chloro substituents is an effective inhibitor although it is still ineffective as a synergist.

If one were to assume that the compounds structurally related to DDT which act as synergists for DDT against DDT-resistant house flies, do so by virtue of their ability to inhibit DDT-dehydrochlorinase it would still be necessary to explain why flies may become resistant to the combinations (March *et al.* 1952). A possible explanation would be that such flies possess a mechanism which attacks the synergist and renders it impotent. DDT-dehydrochlorinase does not attack these synergists regardless of the strain of flies used as a source of enzyme. To propose an additional mechanism seems unnecessary for it has been found that flies resistant to the combination of DDT and synergist may contain amounts of DDT-dehydrochlorinase in excess of those which would result from procedures usually employed to produce selectively a resistant strain with DDT alone. In other words, a comparison of the levels of DDT-dehydrochlorinase in the two types of resistant strains show that the synergist resistant strains contain more DDT-dehydrochlorinase than would be required to protect them from DDT alone (Table 3).

The presence of the enzyme DDT-dehydrochlorinase

Table 3.—DDT-dehydrochlorinase levels in equivalent homogenates of different house fly strains.

STRAIN	HISTORY	μGM. DDE PRODUCED IN 90 MIN. (HOMOGENATE)	LD50 μGM. DDT/FLY (TOPICAL APPL.)
"CSMA"[a]	Susceptible to DDT	0	0.2
"S"[b]	Selected with DDT	410	40.0
"DMC"[c]	Selected with DDT+bis (*p*-chlorophenyl) methylcarbinol	595	>50.0
"Synergist"	Selected with DDT+bis (*p*-chlorophenyl) ethane and bis (*p*-chlorophenyl) chloromethane	660	>50.0

[a] Obtained from Chemical Specialties Manufacturers Association.
[b] Obtained from Dr. W. N. Bruce, State Natural History Survey, Urbana, Ill.
[c] Obtained from Dr. R. W. Fay, U. S. P. H. Savannah, Georgia.
[d] Obtained from Dr. Ralph March, Citrus Experiment Station, Riverside, Calif

in resistant flies and its absence in susceptible flies is the only positive evidence thus far obtained to explain the cause of resistance. It seems justifiable to use this finding as the basis for an explanation of the manner in which house flies which are resistant to DDT but susceptible to DDT plus synergist may be made resistant to the combination. The explanation must lie in the fact that by selection with DDT alone a certain maximum level of resistance will result because DDT alone will not kill enough of the population to exert a further selective effect. With the addition of the synergist, selection can proceed again through a number of generations until maximum resistance to the combination is obtained. In order to explain these phenomena entirely on the basis of DDT-dehydrochlorinase, the synergist-resistant strains would have to contain a higher level of DDT-dehydrochlorinase, which as a matter of fact they do (Table 3). That the so-called DDT synergists do not potentiate the toxic action of DDT for susceptible flies may be explained on the basis that susceptible strains have no measurable amount of DDT-dehydrochlorinase and therefore no synergistic effect should be expected if the single action of the synergist is to inhibit DDT-dehydrochlorinase.

SUMMARY AND CONCLUSIONS.—Certain compounds structurally related to DDT and recognized as synergists for DDT against resistant flies inhibit the enzyme DDT-dehydrochlorinase. Other compounds structurally similar to DDT but lacking a synergistic effect do not inhibit DDT-dehydrochlorinase. One group of inhibitors appears to act competitively with DDT for the enzyme because the enzyme can be wholly reactivated by removing these inhibitors from the reaction mixture. Another group of compounds appears to function in an additional manner because reactivation of the enzyme is not effected when the insoluble portion of these compounds is removed from contact with the enzyme. The enzyme, DDT-dehydrochlorinase, does not attack any of the inhibitors tested regardless of the source of enzyme. It is proposed that the maximum potential level of resistance cannot be obtained by the selective action of DDT alone, because flies attain a level of resistance where DDT will no longer exert a selective effect. The addition of a synergist to DDT will enable selection to proceed through a number of generations until the combination will not kill enough flies in the population to be selective. At this point the flies may have a greater potential for resistance to DDT than when they were selected for DDT alone. This differential may not be detectable by *in vivo* tests. However, if DDT-dehydrochlorinase is a factor in resistance it should be present in larger quantities in the synergist-resistant strains and this has been found to be true. The failure of DDT synergists to potentiate the action of DDT for susceptible flies may be explained in that susceptible flies do not have a measurable quantity of DDT-dehydrochlorinase. Therefore, if the only role of the synergist is the inhibition of this enzyme, then no synergistic effect would be expected. These findings are considered as evidence for the significance of DDT-dehydrochlorinase as an important factor in the cause of house fly resistance to DDT.

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A Comparison of Cyclethrin, Allethrin, Pyrethrins, and Mixtures of Piperonyl Butoxide or Sulfoxide with Them in House Fly Sprays[1]

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With the announcement of the synthesis of a new synthetic pyrethroid, cyclethrin, and of evidence that it is more highly synergized by certain synergists than allethrin (Haynes *et al.* 1954), it became desirable to evaluate this material by the Campbell turntable method with the house fly, *Musca domestica* L., as the test insect. Cyclethrin is the mixture of isomeric esters formed by the acylation of *dl*-2-(2-cyclopentenyl)-3-methyl-2-cyclopenten-4-ol-1-one with a mixture of *cis* and *trans dl*-chrysanthemumic acids, and so differs from allethrin in possessing the cyclopentenyl ring instead of the allyl group in position 2 of the cyclopentenolone nucleus. For simplicity the material will be referred to in this paper as a single compound. It was compared with allethrin and pyrethrins in simple space sprays and in mixed sprays containing allethrin synergists. This paper reports these comparisons.

Materials and Methods.—The only sample of cyclethrin available was a technical product of 82.3 per cent purity. However, according to the manufacturer the remaining material was relatively nontoxic, mortality results obtained by them with an 82 per cent product not differing from those obtained with a 95 per cent product, when corrected for the analyses. Therefore, it appeared practicable to seek information that could be ascribed to cyclethrin without further purification of this sample. It was dark brown, and formed slightly cloudy kerosene sprays from which a very small amount of brownish sediment separated.

The sample of allethrin was of 94 per cent purity as determined by the hydrogenolysis method. The sample of pyrethrins was the complex contained by the complete extractives of pyrethrum flowers without further processing for the removal of materials other than the toxicants; it had been proved to be biologically stable when held in the dark at room temperature. In this sample 61 per cent of the total pyrethrins consisted of pyrethrin I and cinerin I as determined by the mercury-reduction method.

The samples of the synergists used, piperonyl butoxide and sulfoxide, were the technical products obtainable commercially.

Sprays of the three insecticides alone and mixed with a synergist were prepared by dissolving the materials in an appropriate solvent at concentrations desired for stock solutions and by combination and dilution to concentrations selected according to preliminary tests. Refined kerosene was the solvent used for the insecticides and piperonyl butoxide but, because sulfoxide was not completely soluble in that solvent, a mixture of acetone and kerosene was used in the preparation of the sprays containing it. However, the amount of acetone in a spray never exceeded 5 per cent by volume, which had caused no noticeable increase in mortalities in previous work. The mixed sprays were prepared in the proportion of 1 part of insecticide to 10 parts of adjunct.

Knockdown and mortality were determined in replicated tests in each of which approximately 100 flies averaging 2 to 3 days in age were used. All sprays were tested simultaneously on each of six populations of flies.

Table 1.—Mortality and knockdown of house flies caused by kerosene sprays containing cyclethrin, allethrin, or pyrethrins, alone and with piperonyl butoxide or sulfoxide.[a]

Material	Concentration (Mg. per Dl.)			Knockdown in 25 Minutes (Per Cent)	Mortality in 1 Day (Per Cent)
	Insecticide	Synergist	Insecticide Equivalent		
Cyclethrin	300	—	—	100	81.4
	200	—	—	100	58.1
	133.3	—	—	100	42.4
	88.9	—	—	100	25.0
	59.3	—	—	99.5	12.7
Allethrin	200	—	—	100	88.1
	133.3	—	—	100	70.4
	88.9	—	—	100	49.3
	59.3	—	—	100	22.5
	39.5	—	—	100	8.9
Pyrethrins	759	—	—	100	89.0
	506	—	—	100	81.1
	338	—	—	100	65.2
	225	—	—	100	50.9
	150	—	—	100	37.8
	100	—	—	100	15.7
Cyclethrin +piperonyl butoxide	26.3	263	31.3	100	92.6
	17.6	176	20.9	99.7	58.3
	11.7	117	13.9	96.4	20.8
	7.80	78.0	9.29	89.7	6.0
Allethrin +piperonyl butoxide	59.3	593	66.0	100	95.3
	39.5	395	44.0	100	89.1
	26.3	263	29.3	98.8	50.6
	17.6	176	19.6	94.0	14.5
Cyclethrin +sulfoxide	26.3	263	29.6	100	89.1
	17.6	176	19.8	99.7	65.8
	11.7	117	13.2	97.6	20.3
Allethrin +sulfoxide	39.5	395	42.4	100	87.4
	26.3	263	28.3	99.7	50.6
	17.6	176	18.9	97.5	11.3
Pyrethrins +sulfoxide	12.5	125	15.0	100	74.7
	8.84	88.4	10.6	100	43.3
	6.25	62.5	7.49	100	15.5

[a] Means of six replicates.

To evaluate relative toxicity and determine the precision of the estimates, the mortality data were subjected to probit analysis as described by Finney (1952).

Knockdown and Mortality Results.—The knockdown and mortality data are summarized in table 1. Concentrations of cyclethrin are stated in terms of the pure compound. The data show that cyclethrin is less effective than allethrin in both knockdown and mortality caused by simple sprays, but that the cyclethrin mixtures with either adjunct are superior to the comparable allethrin mixtures. The pyrethrins mixture is much more effective than the others with respect to both criteria.

Evaluation of Relative Toxicity and Intensity of Synergism.—To put the relationships on a quantitative basis, provisional lines were fitted graphically to the data in table 1 and weighted individual regression lines

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were computed. No study of parallelism will be given. Comparison of the toxicity of the materials at the 50 per cent mortality level was the principal purpose; therefore, sufficiently precise estimations of the median lethal concentrations (LC-50's) only were sought. The individual lines served this purpose. The concentrations of the sprays were so chosen that the mean concentration for each material was close to the LC-50. Thus, any change in slope of the line would have little effect on the estimate of the LC-50. Actually, it was obvious that the lines for the mixtures were of higher slope than those for the insecticides alone.

The equations of the lines showing the regression of mortality, expressed in probits, on concentration in milligrams per deciliter, expressed as logarithms, are given below. Those for the mixtures are based on the expression of concentration in terms of insecticide content only.

Cyclethrin	$Y=2.8033\ X-1.1445$
Allethrin	$Y=3.6034\ X-2.0996$
Pyrethrins	$Y=2.4462\ X-0.7597$
Cyclethrin+piperonyl butoxide	$Y=5.8229\ X-1.9569$
Allethrin+piperonyl butoxide	$Y=5.6058\ X-2.9728$
Cyclethrin+sulfoxide	$Y=5.9682\ X-2.1386$
Allethrin+sulfoxide	$Y=6.7012\ X-4.5298$
Pyrethrins+sulfoxide	$Y=5.5798\ X-0.4536$

From these equations LC-50's were estimated and relative toxicities were calculated as the inverse ratio of the relevant pair of estimations. The results are reported in table 2. The relative toxicities of the mixtures are given on the basis of insecticide equivalents as well as insecticide content in order to demonstrate synergism and to measure its intensity, since piperonyl butoxide and sulfoxide both possess some toxicity to house flies (Gersdorff *et al.* 1951, 1952). Since no sprays containing the synergists alone were included in this study, the toxicities of these materials relative to allethrin and pyrethrins previously determined by this method (*loc. cit.*) were used. Their toxicities relative to cyclethrin were calculated with the use of the toxicity of the latter relative to allethrin as determined in this study. For piperonyl butoxide these values are: To allethrin, 0.0114 and to cyclethrin, 0.0190. For sulfoxide they are: To allethrin, 0.00751, to cyclethrin, 0.0125, and to pyrethrins, 0.0199.

The insecticide equivalent of a mixture is the sum of the actual amount of the insecticide and the insecticide equivalent of the adjunct. The latter is the product of the actual amount of adjunct and its toxicity relative to the insecticide. To demonstrate synergism a significant increase in toxicity of the mixture on this basis over that expected for similar action (i.e., the toxicity of the insecticide) must be shown. The intensity of synergism is measured by the ratio of toxicity of the mixture on this basis to that of the insecticide.

To determine the requirements for a significant increase in toxicity, estimates of the precision of the measurements were made. The standard errors of the regression coefficients, in the order in which the equations are given, were 0.11, 0.12, 0.15, 0.49, 0.49, 0.77, 0.27 and 0.28. Heterogeneity factors used were, in the same order, 1, 1, 2.975, 6.632, 6.889, 9.222, 1, and 1. The errors of the LC-50's, reported as relative standard errors for simplicity, and the minimum requirements to demonstrate synergism, calculated from the standard errors, are also given in table 2. The standard errors of the ratios of toxicity on the equivalent basis of the mixtures to the corresponding insecticide are also given in columns 7, 10 and 12. The evidence of synergism in each mixture is pronounced.

DISCUSSION OF LETHAL EFFECTS AND CONCLUSIONS.—Cyclethrin was found to be 0.60 as toxic as allethrin and 1.5 as toxic as pyrethrins. It is therefore more toxic than other racemic analogs of allethrin possessing a cyclic substituent in position 2 of the cyclopentenolone nucleus that have been tested in this laboratory (Gersdorff & Mitlin 1954).

Piperonyl butoxide and sulfoxide synergized cyclethrin equally and more strongly than they did allethrin. Sulfoxide synergized pyrethrins more strongly than it did cyclethrin. The intensity of synergism in the cyclethrin mixtures was nearly three times as great as in the allethrin mixtures but a little less than half as great as in the pyrethrins mixture. This information is obtained from the pertinent ratios in table 2 that may be stated in rounded figures as follows: The cyclethrin, allethrin, and pyrethrins mixtures were about $8\frac{1}{2}$, 3, and 20 times as toxic on

Table 2.—Evaluation of relative effects of piperonyl butoxide or sulfoxide in mixtures with cyclethrin, allethrin, and pyrethrins.

MATERIAL	LC-50 (MG. PER DL.)		RELATIVE STANDARD ERROR OF LC-50 (PER CENT)	RATIO OF TOXICITY TO—[a]									LOG OF RATIO ON EQUIVALENT BASIS TO PROPER INSECTICIDE[a]
				Pyrethrins			Allethrin			Cyclethrin			
	Original Basis	Insecticide Equivalent		Original Basis	Insecticide Content	Insecticide Equivalent	Original Basis	Insecticide Content	Insecticide Equivalent	Original Basis	Insecticide Equivalent		
Cyclethrin	155.6	155.6	2.13	1.45	1.45	1.45	0.600	0.600	0.600	1.0	1.0		—
Allethrin	93.4	93.4	1.66	2.42	2.42	2.42	1.0	1.0	1.0	1.67	1.67		—
Pyrethrins	226.2	226.2	3.98	1.0	1.0	1.0	0.413	0.413	0.143	0.688	0.688		—
Cyclethrin +piperonyl butoxide	15.66 +156.6	18.64	3.19	—	14.4	12.1	—	5.96	5.01	—	8.35 ±0.32 (1.08)		0.922 (0.033)
Allethrin +piperonyl butoxide	26.44 +264.4	29.45	3.42	—	8.56	7.68	—	3.53	3.17 ±0.12 (1.08)	—	5.28		0.501 (0.032)
Cyclethrin +sulfoxide	15.71 +157.1	17.67	3.99	—	14.4	12.8	—	5.94	5.28	—	8.81 ±0.40 (1.09)		0.945 (0.039)
Allethrin +sulfoxide	26.43 +264.3	28.41	1.21	—	8.56	7.97	—	3.53	3.29 ±0.07 (1.04)	—	5.48		0.517 (0.017)
Pyrethrins +sulfoxide	9.49 + 94.9	11.38	1.37	—	23.8	19.88 ± 0.84 (1.09)	—	9.84	8.20	—	13.7		1.298 (0.036)

[a] Minimum requirements to demonstrate synergism are given in parentheses under certain ratios and their logarithms.

the basis of insecticide equivalents as the respective insecticides alone, and they were about 12½, 8, and 20 times as toxic as pyrethrins alone.

Often the toxic value of a synergist, especially when it is much lower than that of the insecticide, is ignored in the rating of a mixture for control purposes, For this reason comparisons of the mixtures have also been made based on insecticide content only. Because of the relatively low toxicity of these two synergists, the ratios for the mixtures are increased but little if comparison is made on this basis. The cyclethrin mixtures, the allethrin mixtures, and the pyrethrins mixture are then about 10, 3½, and 24 times as effective as the respective insecticides alone and 14, 8½, and 24 times as effective as pyrethrins alone.

These results show remarkable agreement, insofar as they may be compared, with those reported by Haynes *et al.* (1954). Their tests were made by the large-group Peet-Grady method. Moreover, the amount of synergist in the sprays was kept constant and the amount of insecticide was varied instead of the proportion of insecticide to synergist being kept constant. They determined that to equal the mortality caused by a mixture of 25 mg. of pyrethrins with 200 mg. of either synergist in 100 ml. of refined petroleum distillate, 42 to 50 mg. of cyclethrin and 75 to 100 mg. of allethrin were required. The approximate relative amounts of the insecticides required under these conditions were, respectively, 1, 1.8, and 3.5 in mixtures of 1:8, 1:4, and 1:2 proportion.

In the present study with the amount of synergist kept at 10 times the amount of insecticide in all sprays, the relative amounts of the insecticides required for the mixtures to kill 50 per cent of the flies, as may be calculated from table 2 with the use of means since the effects of the two synergists are not significantly different (e.g., 15.69 divided by 9.49), are in the same order as above, 1, 1.7, and 2.8. Comparison of the 3.5 and 2.8 ratios for the allethrin requirements would indicate that 20 per cent less allethrin is required in the 1:10 than in the 1:2 mixture.

SUMMARY.—A comparison of cyclethrin, allethrin, pyrethrins, and mixtures of piperonyl butoxide or sulfoxide with them was made in space sprays against the house fly, *Musca domestica* L., by the turntable method.

Cyclethrin was 0.60 as toxic as allethrin and 1.5 as toxic as pyrethrins.

In mixtures of 1 part of insecticide to 10 parts of synergist, piperonyl butoxide and sulfoxide synergized cyclethrin equally and more strongly than they did allethrin. Sulfoxide synergized pyrethrins more strongly than it did cyclethrin.

On the basis of insecticide equivalent, the mixtures of the synergists with cyclethrin, allethrin, and pyrethrins were about 8½, 3, and 20 times as toxic as the corresponding insecticides alone, and about 12½, 8, and 20 times as toxic as pyrethrins alone. On the basis of actual insecticide content, the respective mixtures were 10, 3½, and 24 times as effective as the corresponding insecticides alone and 14, 8½, and 24 times as effective as pyrethrins alone.

In sprays containing the insecticide alone cyclethrin was slightly less effective than allethrin in knockdown of flies in 25 minutes.

At comparable concentrations cyclethrin mixtures with either synergist were slightly more effective than the allethrin mixtures in knockdown in 25 minutes.

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Use of Cyclethrin in Livestock Sprays for Control of Flies[1]

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Cyclethrin has been described recently by Haynes *et al.* (1954) as a synthetic compound having many of the attributes of natural pyrethrins. It is a straw-colored viscous liquid having a specific gravity of 1.020 (19% purity) at 20°/20°, and it is compatible with most insecticides and diluents. Cyclethrin was found to show promise against household and field insect pests wherever pyrethrins or allethrins might be used. Approximately 1.5 to 6 times as much cyclethrin was needed as pyrethrins to be effective against a variety of insects. Data indicate cyclethrin had definite advantages over allethrin; in particular it was synergized more readily with existing synergists, such as piperonyl butoxide or sulfoxide.

Because oil and water emulsion formulations containing synergized pyrethrins have been used effectively to prevent biting of flies attacking cattle, it was thought desirable to determine how cyclethrin compared with pyrethrins for this purpose.

METHODS.—Tests were begun in New Jersey in July,

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Table 1.—Repellency tests with oil base sprays[a] against horn flies and stable flies on dairy cattle[b] at New Brunswick, N. J.

Active Ingredients	Mg. per 100 Ml Oil	Average Per Cent Repellency: Day Sprayed	Average Per Cent Repellency: 2nd Day
August 1952			
Pyrethrins Piperonyl butoxide	25 200	88	61
Pyrethrins Piperonyl butoxide plus 5% butoxy polypropylene glycol by volume	15 125	91	65
Cyclethrin Piperonyl butoxide plus 5% butoxy polypropylene glycol by volume	15 125	89	46
Untreated—Avg. no. flies/cow/count, 1st day—20, 2nd day—50			
Pyrethrins	100	86	69
Allethrin	100	93	63
Cyclethrin	100	86	50
Untreated—Avg. no. flies/cow/count, 1st day—35, 2nd day—52			
July 1953			
LSO-1 Pyrethrins Piperonyl butoxide	25 200	90	47
LSO-2 Cyclethrin Piperonyl butoxide	43 200	84	18
LSO-3 Cyclethrin Sulfoxide	43 200	87	9
LSO-4 Pyrethrins Piperonyl butoxide plus 5% butoxy polypropylene glycol	20 160	92	66
LSO-5 Cyclethrin Piperonyl butoxide plus 5% butoxy polypropylene glycol	34 160	88	60
LSO-6 Cyclethrin Sulfoxide plus 5% butoxy polypropylene glycol	34 160	93	29
5% butoxy polypropylene glycol		82	44
Untreated—No. flies/cow/count, 1st day—24, 2nd day—22			

[a] Mentor 28 used as base oil in 1952; Bayol D used as base oil in 1953.
[b] Two ounces of each treatment on 4 cows replicated 3 times at 3–5 day intervals. Brown Swiss, Holstein, and Jersey breeds used.

1952 on Holstein, Brown Swiss and Jersey cows. The susceptibility of the cows to fly attack was determined under pasture conditions prior to spraying, and the cows were arranged in groups of four of equal susceptibility. Each treatment was thus replicated on four cows, and the series of treatments was repeated three times at 3- to 5-day intervals. Approximately 2 ounces of the oil base formulation[5] were used. The sprays were applied by means of a small electric sprayer in the morning immediately after milking, and the cows were then released into the pasture. Counts of horn flies (*Siphona irritans* (L.)) and stable flies (*Stomoxys calcitrans* (L.)) were made at 9:00 and 11:00 A.M. and at 1:30 P.M. A few horse flies (*Tabanus* sp.) were present during part of the season.

In 1953 and 1954 much the same procedure of spraying with the oil base formulations was followed in New Jersey.

In Yonkers, N. Y. the procedure was somewhat different in that the sprays were applied at 3 to 4 P.M., and counts of stable flies were usually made in the morning and afternoon of the following day, approximately 20 and 23 hours after application of the spray. Holstein and Guernsey breeds were used, and 2 to 3 replications at intervals of 2 days were made with the oil sprays applied at the rate of 2 to 3 ounces per animal.

When water emulsions were used in New Jersey, the concentrate formulation was diluted 1 to 9 in water, and 1 quart of the emulsion was applied per animal. As in the tests with the oil formulations, the cows were divided into groups of four. However, because these sprays were effective for a longer period, the three applications were made at intervals of 7 to 8 days. Counts of biting flies, mostly horn flies and stable flies, were made on the day sprayed and for the succeeding 4 days.

Water emulsion sprays at Yonkers, N. Y. were diluted in the same way, and 1 quart was applied per animal. The application was made at 3 to 4 P.M. and counts of stable flies were made on subsequent days. Modifications of

[5] The oil used in 1952 was Mentor 28; in 1953 and 1954 it was Bayol D (Esso Standard Oil Co.).

Table 2.—Repellency tests with oil base sprays[a] against stable flies on dairy cattle[b] at Yonkers, N. Y. August 1953.

Active Ingredients	Mg. per 100 Ml. Oil	Average No. Flies/Cow/Count 20–23 Hours After Spraying	Per Cent Repellency
Series A			
LSO-1 Pyrethrins Piperonyl butoxide	25 200	8.5	50
LSO-2 Cyclethrins Piperonyl butoxide	43 200	9.8	42.3
Untreated		17	—
Series B			
LSO-4 Pyrethrins Piperonyl butoxide plus 5% butoxy polypropylene glycol	20 160	6.6	54.8
LSO-5 Cyclethrin Piperonyl butoxide plus 5% butoxy polypropylene glycol	20 160	6.9	52.7
Untreated		14.6	—

[a] Bayol D was used as base oil.
[b] Two ounces applied to 6 Guernsey and 2 Holstein. Sprays applied twice in Series A and 3 times in Series B, at 3:30 p.m.; counts of stable flies made in morning and afternoon of following day.

these general procedures are indicated in the tables of results.

RESULTS.—In 1952 an oil base formulation containing 0.025 per cent pyrethrins and 0.2 per cent piperonyl butoxide[6] was compared with a formulation in which parts of the synergized pyrethrins were replaced by butoxy polypropylene glycol.[7] A comparable synergized cyclethrin spray was also included in the series. The counts of biting flies taken on untreated cows at the same time as on the treated were used to calculate per cent repellency. The results shown in table 1 indicate that these formulations performed about equally well on the day sprayed. The cyclethrin treatment was somewhat less effective than the other two on the 2nd day.

In another series of oil sprays made later in the same season, pyrethrins, allethrins, and cyclethrins were compared at 100 mg. per 100 ml. of oil. This high concentration was needed because no synergist was used. The results shown in table 1 indicate that the allethrin was slightly superior to the other chemicals on the day sprayed, while pyrethrin was slightly superior on the following day.

On the basis of laboratory Peet-Grady tests against house flies and estimated cost, it was determined that about 1.7 times as much cyclethrin could be used as pyrethrins. On this basis, in 1953 the formulations shown in the LSO number series in table 1 were prepared and tested with the results indicated. At these dosage levels, the pyrethrins and cyclethrin formulations were equally effective. All the formulations provided good repellency on the day sprayed, with a tendency for slightly better protection where butoxy polypropylene glycol and sulfoxide[8] were used with cyclethrin. Best protection was evident on the second day where butoxy polypropylene glycol and piperonyl butoxide were used.

Similar formulations were tested at Yonkers, N. Y. Counts of stable flies shown in table 2, 20 to 23 hours after spraying, indicated that the cyclethrin formulation LSO-2 was slightly less effective than the pyrethrin formulation, LSO-1. Where butoxy polypropylene glycol was used, the difference between pyrethrins and cyclethrins was less marked.

Water emulsion sprays prepared from concentrates of synergized pyrethrins, allethrin and cyclethrin were also tried in 1953. The results of the New Jersey tests are presented in table 3, and results of the New York tests are in table 4. In both localities, the cyclethrin and pyrethrin formulation were about equal in effectiveness, although repellency to the horn fly and stable fly complex was better in the New Jersey tests than in New York where stable flies were the major pests. The allethrin emulsion (Table 3) was slightly less effective in these tests. It was also evident that the oil and emulsifier used in the formulations did not contribute to the repellency of these water emulsion sprays.

Three series of trials were made in 1954, all in New Jersey. One of these was with oil base sprays in which cyclethrin was used at 1.5 and 2 times the concentration of pyrethrins. The concentration of the synergist, either piperonyl butoxide or sulfoxide remained the same (0.2 per cent). In these tests against horn flies, table 5, it appeared that cyclethrin performed best at the higher concentration (0.05 per cent). Sulfoxide at this higher cyclethrin concentration was slightly better as a synergist than piperonyl butoxide.

In the first series of water emulsion sprays in 1954, mainly against horn flies, the concentrations of pyrethrins and cyclethrins in comparable formulations were the same. In these tests, table 6, cyclethrin synergized with either piperonyl butoxide or sulfoxide was slightly better than pyrethrins, approximately 90 per cent repel-

Table 3.—Repellency tests with water emulsion sprays against horn flies and stable flies on dairy cattle[a] at New Brunswick, N. J.

PER CENT ACTIVE INGREDIENTS[b]		AVG. NO. FLIES/COW/COUNT Day Sprayed	2nd Day	3rd Day	4th Day	5th Day	AVG. PER CENT REPELLENCY 4 Days	5 Days
August 1953								
F-1 Pyrethrin	1							
Piperonyl butoxide	10	0.6	3	2	10	31	84	72
F-2 Allethrin	1.72							
Piperonyl butoxide	10	3	3	5	10	32	78	68
F-3 Cyclethrin	1.72							
Piperonyl butoxide	10	1	1	4	10	23	83	76
Oil & Emulsifier		5	11	15	42	62	25	20
Untreated		35	8	18	36	72		
August 1954								
F-26 Cyclethrin	2							
Piperonyl butoxide	8	1	1	2	6	5	89	87
F-27 Cyclethrin	1							
Piperonyl butoxide	5							
Butoxy polypropylene glycol	50	1	1	2	4	5	91	88
F-29 Cyclethrin	1							
Sulfoxide	4							
Butoxy polypropylene glycol	50	1	1	2	4	5	91	88
F-33								
Butoxy polypropylene glycol	50	2	2	4	14	9	76	72
Base oil	85							
Emulsifier	15	6	3	12	14	11	58	57
Emulsifier		23	10	25	32	22		

[a] Sprays applied on four cows replicated 3 times at weekly intervals. Brown Swiss, Holstein, and Jersey breeds used in 1953 and Brown Swiss and Jersey breeds in 1954.

[b] Active ingredients plus 15% emulsifier ("Atlox 1045-A" Atlas Powder Co.) in oil (Bayol D) diluted 1 to 9 in water. One quart of diluted spray applied per animal.

Table 4.—Repellency tests with water emulsion sprays against stable flies on dairy cattle[a] at Yonkers, N. Y. July 1953.

PER CENT ACTIVE INGREDIENTS[b]		AVG. NO. FLIES/COW/COUNT DAYS AFTER SPRAY 1	2	3	4	5	AVERAGE PER CENT REPELLENCY 5 DAYS
F-1 Pyrethrins	1						
Piperonyl butoxide	10	4.6	5.0	6.1	5.5	7.8	61
F-3 Cyclethrin	1.72						
Piperonyl butoxide	10	3.4	6.2	7.8	7.9	8.8	54
Untreated		14.3	15.5	16.3	10.6	18.0	

[a] Sprays applied to 7 cows, 2 Holstein and 5 Guernsey.

[b] See note b, Table 3.

[6] U. S. Industrial Chemicals Co., now Fairfield Division, Food Machinery Corp.

[7] Carbide and Carbon Chemicals Co.

[8] S. B. Penick & Co.

Table 5.—Repellency tests with oil base sprays against horn flies on dairy cattle[a] at New Brunswick, N. J. July 1954.

Active Ingredients[b]	Mg. per 100 Ml. Oil	Average Per Cent Repellency		
		Day Sprayed	2nd Day	3rd Day
LSO-21 Pyrethrins	25			
Piperonyl butoxide	200	90	71	37
LSO-22 Cyclethrin	37			
Piperonyl butoxide	200	88	50	21
LSO-23 Cyclethrin	50			
Piperonyl butoxide	200	90	63	56
LSO-24 Cyclethrin	37			
Sulfoxide	200	89	42	24
LSO-25 Cyclethrin	50			
Sulfoxide	200	94	77	65
Untreated, Ave. No. flies/cow/count		35	41	41

[a] Bayol D used as base oil.
[b] Two ounces of each treatment applied on 4 cows replicated 3 times at 3–5 day intervals. Brown Swiss and Jersey breeds used.

lency being obtained over a 4-day period. These results are comparable with those obtained in the laboratory when residues of the chemical were tested after application to plywood panels. Cyclethrin tended to remain toxic to house flies over a longer period than pyrethrins.

In the second series of water emulsion sprays in 1954, table 4, against both horn flies and stable flies, a 2 per cent synergized cyclethrin concentrate was compared with formulations in which half the cyclethrin was replaced with 50 per cent butoxy polypropylene glycol. These sprays provided excellent protection (about 90 per cent repellency) for 4 and 5 days. In comparison, an emulsion spray from a concentrate containing 50 per cent butoxy polypropylene glycol without any toxicant was about 75 per cent repellent over this period, and one from a concentration of oil and emulsifier alone was about 58 per cent repellent.

Other Applications.—Cyclethrin has been tried in automatic treadle sprayers using the F-3 formulation shown in table 3 (Granett *et al.* 1955), and one containing 0.5 per cent cyclethrin plus 2.0 per cent piperonyl butoxide and 50 per cent butoxy polypropylene glycol in oil. These formulations provided good protection against horn flies and stable flies, and they compared favorably with similar formulations containing synergized pyrethrins.

Table 6.—Repellency tests with water emulsion sprays against horn flies on dairy cattle[a] at New Brunswick, N. J. June, July 1954.

Per Cent Active Ingredients[b]	Avg. No. Flies/Cow/Count					Avg. Per Cent Repellency	
	Day Sprayed	2nd Day	3rd Day	4th Day	5th Day	4 Days	5 Days
F-30 Pyrethrins 0.5 Piperonyl butoxide 5.0	1	1	2	3	18	83	71
F-31 Cyclethrin 0.5 Piperonyl butoxide 5.0	.5	.7	1.5	2.5	13	89	80
F-32 Cyclethrin 0.5 Sulfoxide 5.0	1.5	.2	.5	2	9.5	90	84
Untreated	10	3.2	11	18	45		

[a] See note a, table 3. Brown Swiss and Jersey breeds used.
[b] See note b, table 3

Summary and Conclusions.—Cyclethrin, a new synthetic pyrethrin-type insecticide, has been tried against horn flies and stable flies on dairy cattle. Synergized with piperonyl butoxide or sulfoxide in an oil base spray used at the rate of approximately 2 ounces per animal, it was found that 1.5 to 2 times as much cyclethrin is needed as pyrethrins, to provide effective repellency on the day sprayed. When water emulsion sprays were used and 1 quart of the diluted concentrate was applied per animal, effective repellency was obtained for 3 to 5 days from concentrations equivalent to pyrethrin formulations. Part of the toxicant in the spray could be replaced with butoxy polypropylene glycol, generally with an increase in the effective protection period.

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The Effect of Pyrethrins in Combination with Chlorinated Hydrocarbons on Resistant and Nonresistant House Flies[1]

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A DDT resistant strain of house flies being reared for insecticidal tests exhibited a high degree of resistance to all chlorinated hydrocarbons to which it had been exposed. Results obtained from use of the OTI (Official Test Insecticide) in the Peet-Grady chamber indicated that this strain also showed a marked degree of resistance to pyrethrins. Though the knockdown rate was only slightly lower than that for nonresistant flies, the resulting mortality was considerably lower. Since pyrethrins and chlorinated hydrocarbons are quite dissimilar both in structure and mode of action, it was thought that the toxicity of combinations of these substances might provide some useful information. To provide an additional point of comparison flies from both the DDT resistant and nonresistant cultures were exposed to these combinations.

METHODS.—The chlorinated insecticides used were aldrin, dieldrin, endrin, isodrin, chlordane and heptachlor. These were first tested alone and then in combination with pyrethrins. All formulations were calculated in per cent concentrations on a weight-weight basis in deobase. The concentration for each compound was calculated to give a mortality of less than the LD-50 value for the particular compound when run alone against DDT resistant flies. This was accomplished by using LD-50 values for the resistant strain as determined previously on a weight-volume basis and formulating these values on a weight-weight basis. Thus the per cent value remained the same but, due to the low specific gravity of deobase, the amount of toxicant per unit volume was reduced by the same factor in each case. The actual decrease in mortality for any particular compound depended upon the characteristic dosage-mortality curve for this compound.

The test insects consisted of two strains of the house fly, *Musca domestica* L.; one strain was DDT resistant and the other nonresistant. The DDT resistant flies were of the Orlando-Beltsville strain obtained in 1952. The normal or nonresistant flies were the official C.S.M.A. strain of 1951.

Both strains of flies were reared according to the official Peet-Grady method as described in Soap Blue Book (1952) except for minor changes necessary for mold control and for maintaining the level of resistance to DDT. Mold formations on the jars of larval media were controlled by adding a layer of sand 1 inch thick to the top of the media 48 hours after the jars were seeded with eggs. Since the maggots pupate in this sand layer, they were easily collected by sifting the sand through a sieve which retained the pupae (Born 1954). A high level of resistance was maintained by keeping the adults of each generation in a special breeding cage. This cage had all inner surfaces treated with a 50 per cent acetone solution of DDT painted on with a brush. All eggs used for rearing this strain were collected from this cage.

The tests were conducted using a turntable modification of the Peet-Grady chamber (Lockard & Fisk 1953).

Table 1.—Comparative mortalities of compounds alone, in combination with pyrethrins and the expected combined mortalities.

COMPOUND	AV. PER CENT MORTALITY OF COMPOUND ALONE	AV. PER CENT MORTALITY OF THE COMBINATION	CALCULATED PER CENT MORTALITY[a]
	DDT Resistant Strain		
Dieldrin	33	56	48
Aldrin	19	36	34
Heptachlor	12	28	27
Chlordane	25	55	40
Isodrin	8	20	23
Endrin	4	18	19
	Nonresistant Strain		
Dieldrin	57	85	77
Aldrin	28	56	48
Heptachlor	73	48	93
Chlordane	36	64	56
Isodrin	53	40	73
Endrin	24	48	44

[a] The per cent mortality of the compound alone plus the per cent mortality of pyrethrins alone.

This differs from the standard Peet-Grady chamber in that a turntable is placed in the center of the chamber. The flies are tested in cylindrical screen cages fastened to the turntable and revolved at 28 r.p.m. while the test is in progress. As many as four cages may be tested at one time. A total of 12 milliliters of insecticide is sprayed through the 8 ports located near the corners of the chamber. This modification permits the use of materials having little or no knockdown effect.

Each test consisted of the test compound replicated six times for each strain of house fly and the standard insecticide replicated twice for each strain. The OTI was used as a standard in all tests. Due to the limitations imposed by time and equipment, only 1 compound could be tested in any 1 day. Therefore, the OTI served both as a check on the reliability of testing conditions and as a means of evaluating the data taken on different days.

Test insects were inactivated by placing them in a refrigerator at 40° F. for 30 minutes to facilitate transfer to the testing cages. Approximately 100 flies were placed in each testing cage. Four cages of insects were tested at a time, two cages of DDT resistant and two cages of normal flies. The turntable was set in motion and the insecticide applied in spray form through the ports. The flies were left in the spray-filled chamber for 10 minutes after which time the chamber was evacuated for 5 minutes. The cages were then removed and the flies transferred to clean recovery cages of the same design as the testing cages. Each recovery cage contained a ball of cellucotton soaked in sugar water to provide food and moisture during the holding period. The holding room was

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kept at a constant temperature of 80° F. with an average humidity of 36 per cent. Continual illumination was provided during the 24 hour holding period to insure maximum activity of the flies.

Knockdown data was recorded at 10 and 20 minutes after applying the insecticide. Mortality was recorded after 24 hours by counting the number of dead flies on the bottom of each cage. The cages were then placed in an autoclave for about 1 minute to kill the remaining flies. The total number of flies in each cage was then counted and the percentage mortality calculated.

RESULTS.—The results are summarized in table 1. Two cases of possible antagonism were encountered in combinations with pyrethrins. In both instances the effect was noticed only when the compounds were tested on nonresistant flies. The same combinations when tested on DDT resistant flies resulted in joint action. Heptachlor, when run alone against nonresistant flies, gave an average mortality of 73 per cent. When this same concentration of heptachlor was combined with pyrethrins the average mortality on nonresistant flies dropped to 48 per cent. The tests with isodrin produced similar results. Isodrin alone on nonresistant flies caused a mortality of 53 per cent, but when combined with pyrethrins this kill was reduced to 40 per cent. This last is not a great difference and could be accounted for within the limits of experimental error, but only if the addition of pyrethrins was considered to have no effect except possibly as a diluent.

All other tests resulted in combined joint action where the mortality obtained from the two compounds in combination was, within the limits of variation, equal to the sum of the mortalities obtained from the component compounds when tested alone. Synergistic action was not observed in any of the combinations used.

SUMMARY AND CONCLUSIONS.—Aldrin, dieldrin, endrin, isodrin, chlordane and heptachlor were evaluated alone and in combination with pyrethrins on both DDT resistant and C.S.M.A. nonresistant house flies. The tests were conducted using a turntable modification of the Peet-Grady method.

All of the compounds gave increased mortality when combined with pyrethrins and tested against flies of the DDT resistant strain. This increase was, within the limits of experimental error, equal to the sum of the mortalities of the components tested alone. When the nonresistant strain was used the results were additive for all compounds except heptachlor and isodrin. These two compounds gave indications of antagonism in combination with pyrethrins.

The apparent antagonism exhibited by heptachlor and isodrin may be due to physiological differences between the two strains of flies since there was no evidence of antagonism on flies of the resistant strain. It would seem, therefore, that the DDT resistant strain may show a higher degree of susceptibility to some particular combinations of toxicants than does the nonresistant strain. Further investigation is necessary to definitely prove this.

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Mexican Fruit Fly and Citrus Blackfly Control in Mexico[1]

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The Plant Pest Control Branch of the Agricultural Research Service is conducting two campaigns in Mexico in cooperation with the Mexican Department of Agriculture against two serious citrus insects. Its efforts are being directed against the Mexican fruit fly, *Anastrepha ludens* (Loew), and the citrus blackfly, *Aleurocanthus woglumi* Ashby. Inasmuch as this report deals with the progress of these two programs, the campaign against each will be discussed separately.

CITRUS BLACKFLY SURVEYS.—The purpose of citrus blackfly surveys in the northern states of the Republic of Mexico is to discover incipient infestations of this insect before the infestations become so widespread that eradication would be impossible, and the fly would eventually spread into the citrus producing areas of the United States.

The Plant Pest Control Branch is concerned solely with assisting the Mexican cooperating agency in locating the infestations. Under this program all control and eradication activities, as well as road station operations, are the responsibility of the Mexican National Blackfly Committee.

As early as 1949 the citrus blackfly was causing serious injury to citrus plantings over a large part of Mexico and as far north as Valles in San Luis Potosi, about 350 miles south of the International Border. It was feared that the pest would soon become established in the citrus groves in northern Tamaulipas and Nuevo Leon. If this occurred it would be a simple matter for the fly to spread to Texas. For that reason, Texas citrus producers were extremely anxious that a campaign be undertaken jointly with our neighbors to the south to see if the steady and rapid northward movement of this insect could be stopped. Accordingly, arrangements were made whereby the Plant Pest Control Branch, then part of the Bureau of Entomology and Plant Quarantine, would assist in locating incipient infestations to the extent of furnishing transportation facilities, supervisory personnel and paying the salaries of some of the inspectors.

The program got underway in January 1950. Infesta-

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tions were discovered with discouraging regularity in the city of Monterrey, in the groves in the Montemorelos area, and one infestation was found even as close to the Border as Matamoros. Fortunately, the National Blackfly Committee was well organized and had competent personnel and excellent spray equipment available for applying eradication sprays to the trees on infested properties as rapidly as they were discovered by the survey crews. As a result of this prompt action no infestation got out of hand, even though during the succeeding year over 200 light infestations were found in the area from Victoria northward throughout the state of Nuevo Leon, and four were discovered in Hermosillo in the state of Sonora in northwestern Mexico.

Evidence of the effectiveness of the work became apparent in the following year when only 65 infestations were discovered in the state of Nuevo Leon, and only two in northwestern Mexico. The first infestation found in Matamoros, just across the Rio Grande from Texas citrus plantings, in January 1950, was apparently eradicated as no other citrus blackflies could be located in 1951. But in late 1952 the blackfly was again discovered in Matamoros and before the inspection was completed it was found on more than 100 dooryard plantings. Almost the same conditions prevailed in Victoria, about 200 miles south of the Border, where the pest was found widely distributed on a few trees on many properties.

Again, prompt and efficient action by the spray crews of the National Blackfly Committee eradicated the infestation in Matamoros and so greatly reduced the fly population in other localities that subsequent inspections revealed live blackfly forms on only a few properties. In Monterrey, the application of sprays has been exceedingly effective. Continued inspections have resulted in the discovery of only an occasional infested leaf on a limited number of dooryard properties.

In addition to the neaby infestations found at Matamoros, the blackfly was discovered in Sabinas Hidalgo in late 1950 and again in 1954. At Nuevo Laredo, again on the Border, the blackfly was not discovered for the first time until the summer of 1954, even though intensive inspections had been made there annually ever since the program was inaugurated.

The citrus blackfly can and undoubtedly does fly from one location to another. But from experience gained in making inspections it is apparent that three other methods of distribution have been largely responsible for the wide and rapid spread of this pest throughout the Republic. In addition to flight, mentioned above, one method of dispersal is through the shipment of infested foliage in packages or bulk loads of fruit; another is by the movement of infested nursery stock, while the last, and probably most important, is the accidental transportation of flies in motor vehicles. Repeated inspections in cities, along highways, and at other places where people congregate and cars are stopped, have proved that people carry blackflies. For this reason, preventing the blackfly from getting out of hand in the cities in the states of Tamaulipas, Nuevo Leon, Sonora and Baja California is of extreme importance to everyone interested in controlling this pest. The possibility that motorists will bring the blackfly with them when coming from Mexico becomes almost a certainty, unless the infestation in these states can be kept to a point where this pest is difficult to find even by trained survey crews.

This cooperative survey program has been as beneficial to the Mexican citrus producers as to the North American fruit growers. In northern Tamaulipas, Nuevo Leon, Sonora and Baja California, where surveys are being made and the blackfly kept under control, citrus growers are producing excellent quality fruit that is free of any characteristics of fruit produced in heavily infested plantings. Producers in these states can compete with other growers on the home or international markets on an equal basis, and receive a premium for their fine product. By preventing this insect from invading the groves north of the International Border similar beneficial results have accrued to the North American citrus growers.

Although the principal cooperating agency in Mexico is the National Blackfly Committee, invaluable technical assistance on this program has been received through the years from the research group currently designated as the Fruit Insects Section, Entomology Research Branch, Agricultural Research Service. This agency conducted large scale spray control demonstrations in groves of northeastern Mexico for several years and in the Guaymas-Empalme and other points on the West Coast of Mexico. The Section had the responsibility for the conduct of eradication of citrus blackfly operations in the Hermosillo, Sonora area until that activity was taken over by the National Blackfly Committee recently. The Defensa Agricola of the Mexican Department of Agriculture has given appreciable support to the program in many ways.

MEXICAN FRUIT FLY.—Control of the Mexican fruit fly presents a different set of problems from those involved in preventing the blackfly from becoming established in northern Mexico and southwestern United States. Even before the Rio Grande Valley of Texas was opened to settlers from the northern part of the United States, the Mexican fruit fly was known to be infesting citrus fruits in various parts of Mexico. When this fly was found in Texas grapefruit plantings in 1927, it was thought the infestations could be promptly eradicated. A campaign was undertaken to accomplish this. It developed, however, that eradication was not possible because each fall and winter large numbers of flies move from northeastern Mexico to southern Texas and establish infestations in Texas citrus plantings. Although there was no known method of eradicating the fly in Mexico, nor of preventing its flight across the Rio Grande, it was believed that the fly could be prevented from spreading from Texas to other parts of the nation and a very strict quarantine was promulgated. All fruit moving from the infested area in Texas has continued to be shipped under the provisions of this quarantine from 1927 to date. The quarantine is extremely successful, as the Mexican fruit fly has not spread from Texas to other fruit growing sections of the nation.

Growers in other fruit growing sections, particularly in Arizona and California, have been concerned lest this fly become established in northwestern Mexico. When fruit fly traps revealed that the fly was present in Tijuana, Baja California, it was only natural that those growers should become greatly alarmed. They were fearful, and rightly so, that the Mexican fruit fly would spread from

the northern part of Baja California and Sonora across the International Border and begin infesting not only their citrus but deciduous fruits as well.

Between January and October, 23 Mexican fruit flies were trapped on 11 properties in Tijuana, one at Mexicali and one at Ensenada, Mexico. In addition, one fly was taken across the line in San Ysidro, California. Of the 17 female flies trapped, only two of them have shown any signs of egg development, and in spite of very intensive fruit inspections no larval infestations have been found. It is interesting to note, however, that soil sifting under a sapote tree on one of the properties in Tijuana revealed that a larval infestation had been established there, probably before the first fly was trapped, as three empty puparia were collected.

Because of the trapping of fruit flies on both sides of the International Border in California and Baja California, an aggressive eradication campaign is now being conducted. All host trees within the area are being sprayed at 21-day intervals with a bait spray composed of brown sugar, tartar emetic and water. In addition, fruits are being closely inspected and grove sanitation is being rigidly enforced. Fortunately, within the infested area there are no large quantities of host fruit produced, but there are several hundred dooryard plantings which have to be sprayed and on which the fruit needs to be periodically inspected. Although there are some serious problems to contend with in carrying out such a program, it is believed that the Mexican fruit fly can be eradicated from this area.

This opinion is based upon the fact that whereas in northeastern Mexico there is a wild host which supplies a constant population of flies, some of which each year migrate to Texas, so far as is known there is no wild host on the West Coast of Mexico close to the International Border. The only means of maintaining an infestation in that area, in the presence of an intensive spray campaign, is through the introduction of infested fruit. Therefore, if infested fruit can be prevented from being shipped to cities in northern Baja California, it is believed that the proper application of poison sprays and the collection of fallen host fruits will bring about eventual eradication. Prevention of the shipment of infested fruit from the mainland to the Northwest territory does not present a problem too difficult to solve. While Mexico produces many delicious fruits, several of which are hosts of the Mexican fruit fly, the one fruit that is probably the favorite not only of the Mexicans, but the Californians as well, is the Manila mango. It is also a preferred host of the Mexican fruit fly. There is a ready market for this type of fruit in northern Sonora and Baja California and unless ways and means are provided for supplying this demand legally, it is only logical to presume that mangoes will be brought into the area illegally. This occurred last season. Infested mangoes were found on the markets at both Tijuana and Mexicali.

Recently arrangements were made whereby Manila mangoes could be safely shipped from the interior of Mexico to the area along the International Border. A method of fumigating fruit with ethylene dibromide has been developed which is an effective killer of the larvae of the Mexican fruit fly. Last spring a fumigation chamber was set up at Hermosillo and all mangoes were fumigated before being shipped to the markets at Tijuana, Mexicali, Ensenada and Nogales.

As a result of the curtailment of the shipment of infested fruit into northern Baja California, and because of the intensive spray campaign which is being conducted in that area, it is anticipated that the fruit fly population will be entirely eliminated there in the very near future. It then remains primarily a quarantine problem to prevent the same conditions which resulted in this present infestation from recurring. It is expected that the continuation of effective cooperation on the part of Mexico, the United States, Arizona and California in the program just described will accomplish the desired results and that the Mexican fruit fly will be prevented from becoming established in northwestern Mexico or southern California.

The Effect of Particle Size of the Active Ingredient in DDT Dust on Corn Earworm Control[1]

JOHN W. WILSON and WALTER H. THAMES[2]

DDT, in dust formulations or as an emulsifiable concentrate alone and combined with mineral oil, has become the standard insecticide against the corn earworm, *Heliothis armigera* (Hbn.), on sweet corn. The fresh corn market demands a fancy, unclipped, worm-free product. In areas where sweet corn is grown principally for the fresh market and where earworm populations are usually very high during the latter part of the growing season, DDT has not been entirely satisfactory as a control measure, either in dust or liquid form.

In a review of the literature on the relationship between particle size and insecticidal efficiency, Smith & Goodhue (1942) have shown that there is considerable experimental evidence to support the view that the effectiveness of dust formulations increases as the particle size decreases. Steiner *et al.* (1944) prepared a series of DDT pastes having mean surface particle diameters ranging from 9.2 to 4.3 microns. Applied as water suspensions in laboratory tests against codling moth larvae, the DDT having a mean surface particle diameter of 4 to 5 microns was more effective. But the ability of the deposit to withstand the weathering effects of artificial rain diminished as the di-

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[2] Central Florida Experiment Station, Sanford, and Everglades Experiment Station, Belle Glade, respectively.

Table 1.—Particle size specifications of dusts used in 1953.

PARTICLE SIZE BELOW	DDT IN 5% COMMERCIAL FORMULATION	SPECIAL FORMULATIONS					
		Method Grinding DDT			Diluents		
		Hammer Mill	Roller Mill	Air Mill	No. 1	No. 2	No. 3
	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent
80-mesh	100.0						
100-mesh	92.4	100.0	100.0				
200-mesh	69.5	90.0	98.0	100.0	100.0		
325-mesh	63.0	77.7	90.0	99.0	94.7	100.0	100.0
40 microns	52.4	51.8	68.5	98.5	86.7	96.5	—
20 microns	11.9	36.0	40.0	94.8	65.2	77.9	—
10 microns	8.7	19.8	23.4	85.0	31.6	45.0	97.2
5 microns	3.2	13.5	16.4	46.1	2.0	20.0	84.1
2 microns	—	—	—	—	—	—	68.4
1 micron	—	—	—	—	—	—	54.3
0.5 micron	—	—	—	—	—	—	44.1
Mean surface particle diameter in microns[a]	20.3	11.5	9.8	4.1	12.5	7.0	0.5
Per cent of final air milled formulation[b]					36.0	31.0	23.0
Per cent of final roller and hammer mill formulations					38.5	31.0	25.5
Mean surface particle diameter of finished dusts in microns	1.75	1.8	1.8	1.8			

[a] Figures in the first 4 columns refer to the particle size of the DDT, and in the last 3 columns to the particle size of each of the diluents.
[b] The air milled DDT was purchased and contained 95% DDT and 5% diluent of unknown specifications.

ameter was reduced below 9 microns. In field experiments for the control of the leafhopper, *Erythroneura elegantula* Osb. on grapes, Frazier & Stafford (1947) used DDT having mean surface particle diameters of 3, 5 and 10 microns in several mixtures, with dusting sulfur as the diluent. The difference in the particle size of the DDT in these mixtures did not appreciably affect the degree of control obtained.

Because the mean surface particle diameter of the materials usually used as diluents in insecticidal dusts is 10 microns or smaller (Watkins & Norton 1947) and the mean surface particle diameter of the DDT used in commercial formulations is usually about 20 microns or larger (Mayeux 1953), it seemed advisable to investigate the effectiveness of dusts formulated from DDT ground to a smaller particle size. This paper reports the results of comparisons during two seasons in central and south Florida of dusts made up with DDT ground in three types of mills to produce DDT of a medium, fine and very fine mean surface particle diameter. In this paper the DDT produced by the hammer mill, roller mill and air mill has been designated as medium, fine and very fine DDT, respectively.

Table 2.—Amounts of dusts applied per acre for each treatment.

TREATMENT (DDT)	POUNDS PER ACRE APPLIED		
	Sanford		Belle Glade
	1953	1954	1954
Commercial	34.3	29.9	—
Medium	32.0	28.4	30
Fine	36.4	31.5	36
Extra Fine	30.5	28.5	31

MATERIALS AND METHODS.—The commercial 5% DDT dust used at the Central Florida Experiment Station was purchased from a local insecticide dealer. A sample of this dust analyzed by the laboratory of the Florida Agricultural Supply Company was found to have a mean surface particle diameter of 1.75 microns, the DDT in the dust being 20.3 microns.

The three special 5% DDT dust formulations were prepared by The Florida Agricultural Supply Company. The particle size specifications of the materials used during the 1953 crop season are given in table 1. The mean surface particle diameter of the three finished special DDT dusts was 1.8 microns and of the commercial dust, 1.75 microns. The mean surface particle diameter of the DDT used in the dusts formulated for the 1954 season were: medium DDT, 11.5 microns; fine DDT, 9.5 microns; very fine DDT, 4.0 microns. The particle sizes of the dusts used in 1954 resemble so closely the specifications of the 1953 dusts that the detailed figures have not been included in table 1.

Ioana sweet corn planted to mature near or after May 15 when earworm populations are expected to be high was used for four of the five tests. Golden Security was planted for the 1954 test at Belle Glade. The plots were arranged in simple randomized blocks with 4 to 6 replications. During the growing period prior to silking, the corn was sprayed four times with 1 quart of 25% emulsifiable DDT in 100 gallons of water per acre for the control of fall armyworm, *Laphygma frugiperda* (J. E. Smith), and corn earworm larvae. The last of these applications was made 4 to 6 days before the appearance of the first silks.

Table 3.—Effect of DDT particle size in dust formulations on corn earworm control.

DDT Treatment	Per Cent Worm-Free Ears Harvested				
	Sanford		Belle Glade		
			1953		
	1953	1954	Test No. 1	Test No. 2	1954
Commercial	69.2	44.7	—	—	—
Medium	70.0	58.0	73.7	70.8	73.2
Fine	78.3	52.0	75.0	64.0	75.4
Extra Fine	79.2	70.0	87.0	65.6	66.6
Untreated	1.7	7.5	18.3	0	14.0
L.S.D. at 5% level	17.0	25.5	11.9	10.2	10.1

A rotary-type hand duster, calibrated to deliver 30 pounds per acre, was used to apply the dusts. The amount of dust used for each application was recorded for all except the two 1953 trials at Belle Glade. The amounts applied per acre were calculated and the averages of these figures are given in table 2. The first application was made at Sanford the day after the first silks appeared and at Belle Glade when about 10 per cent of the ears were in silk. At Sanford, a total of six applications and at Belle Glade, five applications, spaced at 48-hour intervals, were made.

The degree of control obtained at Sanford was calculated from the number of worm-free ears in a sample of 25 primary ears taken at random from the two center rows of each plot. At Belle Glade, all of the primary ears from the center row of each plot were examined. The percentage of worm-free ears was calculated from the results of these examinations.

RESULTS.—Results of the five tests are presented in table 3. The particle size of the DDT in the dust formulations used did not affect the control of the corn earworm. Only two of the five tests showed a slight indication of increased earworm control with decreasing particle sizes of DDT.

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Experiments in the Technique of Artificial Insemination of Queen Bees[1]

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Since 1935 a number of improvements in the technique of artificial insemination of queen bees have been made at the Southern States Beekeeping Laboratory, at Baton Rouge, La. In 1948 a manual was published (Mackensen & Roberts 1948) describing a method and the results to be expected, and in later papers (Mackensen 1948, 1954) some improvements were described. The purpose of this paper is to report various experiments made during the development of the technique.

METHOD.—In general the method of insemination was as described in the manual. The sperm was injected into the oviducts beyond the valvefold while this structure was lowered with a probe. A modified pencil-type syringe (Nolan 1937) was used. The syringe tip consisted of a glass tube fitted to a brass wire plunger (27-gage, 0.014 inch in diameter). The end of the tip tapered to an outside diameter of about 0.010 inch and an inside diameter of not less than 0.006 inch. The glass tip and plunger were attached to a mechanical pencil, and the plunger was operated by the mechanism of the pencil. To prepare the syringe for use, water was forced around the plunger. A short column of water was left at the end of the plunger and then a short air space before the sperm was drawn in. The air space helped to prevent mixture of water with the sperm or semen.

In the early work sperm was taken into the syringe from the seminal vesicles. A window was cut into the dorsal side of the abdomen, and the vesicles were carefully cut off at the junction with the mucus glands. They were carefully laid out on a finger, and caused to contract peristaltically by squeezing the upper end with forceps. The droplet of sperm at the cut end was then taken into the syringe.

Sperm is now taken from the completely everted penis. By this method the penis is made partially to evert by pressure on the abdomen or by exposure to chloroform fumes. During this process the sperm and mucus are ejaculated into the penis bulb, where the sperm mixes with the fluid of this structure and possibly with some of the soft mucus to form semen. Eversion is completed by further pressure on the abdomen until the semen and mucus appear. Practically all the semen can be taken into the syringe without noticeable addition of mucus. This is by far the better method, and is now used exclusively in routine inseminations. In this paper the fluids used in this method will be called semen and the material taken directly from the seminal vesicles will be called sperm.

Various modifications and variables were introduced, as indicated in the different experiments, but all queens

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in each experiment were inseminated identically and treated as nearly the same as possible except for the particular variables under test.

The clear fluid in the penis bulb was often utilized when sperm was taken from the vesicles. This was done by simply cutting a hole in the bulb and taking the fluid up into the syringe. Usually the sperm was taken into the syringe first, followed by bulb fluid from the same drone. Sometimes fluid was taken from one drone, followed by the sperm and the fluid from a second drone.

Mucus was sometimes applied as a plug in an attempt to prevent loss of sperm or semen from the reproductive tract. For this purpose a syringe of a uniform inside diameter (0.014 inch) was used so that the plunger could be pushed to the end. This syringe could be inserted into the vagina but was too large to enter the oviduct. After completion of an insemination about 0.2 mm.^{3} of mucus was taken into this syringe and injected into the vagina. This amount barely filled the vagina, and when the sting was released there was no mucus lodged between the vaginal opening and the sting. Often a fresh drone provided the mucus. Sometimes the opening of the reproductive tract was simply smeared with mucus, which was then held in place by the sting when released. The smear was applied by squeezing a dissected mucus gland with forceps.

In the early work no anesthetic was used on queens, but since 1943 carbon dioxide has been used. In 1944 it was found to stimulate initial oviposition in artificially inseminated and virgin queens after two exposures. It was made to pass through the queen holder, thus bathing the queen. If no insemination was desired, the queens were simply exposed in a jar into which the gas was flowing.

When sperm was taken from the seminal vesicles, a volumetric measure, cubic millimeters, was considered the most accurate. This measure was carried over when the change was made to semen collection by complete eversion. Additional drones were utilized until the desired measure of sperm or semen was obtained.

Unless otherwise noted, queens and drones were kept in cabinets at constant temperature and humidity. Queen cells and drone brood were placed in a cabinet kept at 35° C. and about 75 per cent relative humidity. As the individuals emerged they were transferred to a cabinet kept at 33° and a relative humidity of 25 to 30 per cent. Queens were kept in individual cages together with a few worker bees, or in small cages placed on the screen top of a large cage of worker bees so that the workers could feed the queens through the screen. Drones were also kept in the dry cabinet in cages with at least an equal number of worker bees. Water, sugar sirup, and pollen were provided. The low humidity prevented excessive accumulation of feces, which interfered with insemination. Examination for degree of insemination was delayed until at least 1 day after the last insemination.

The success of an insemination was measured by one of two methods. In the opacity method a permanent standard series of 10 spermathecae, preserved in alcohol, was numbered according to the amount of light transmitted, from the least to the most opaque. Since the opacity increased with the number of sperms present, any spermatheca dissected could be compared with this series and given the appropriate number. To compare the groups the numbers for the spermathecae in each group were averaged. In the counting method the sperms were dispersed in 10 cc. of tap water with the aid of a medicine-dropper pipette. Those in a 0.8 mm.^{3} sample of this dilution were counted with the aid of a counting-chamber slide, and the total calculated. This method is described by Mackensen & Roberts (1948).

NUMBER OF SPERMS IN MATURE HIVE-REARED DRONES.—Eight drones caught at the hive entrances as they returned from the field were found to contain in their seminal vesicles an average of 10.41 million sperms, ranging from 9.34 to 11.97 million.

SEXUAL MATURITY OF DRONES.—Drones were kept in the low-humidity cabinets. They were 1 day old or less at time of caging. Each day six drones were removed and the sperms counted. The numbers of sperms (millions) in drones at various ages are given below.

Age in Days	*Range*	*Average*
3– 4	0.005– 0.34	0.13
4– 5	3.45 – 6.37	5.15
5– 6	7.66 – 9.50	8.69
6– 7	8.28 –10.38	9.14
7– 8	8.36 –10.63	9.89
8– 9	8.63 –10.53	9.65
13–15	8.56 –10.23	9.21

These results show that drones 3 to 4 days old can be utilized for artificial insemination if necessary; however, the sperm must be taken from the seminal vesicles, since at this age drones do not ejaculate. Drones 7 to 8 days old contained nearly as many sperms as were found in mature hive-reared drones.

NUMBER OF SPERMS IN SPERMATHECAE OF NATURALLY MATED QUEENS.—Data are available from 33 queens which mated during 1942 and 1943. They were of various strains and were reared at various times of the year. All were taken from mating nuclei, and none had been laying more than a month when the sperm counts were made. This is the same group of queens mentioned in the manual (Mackensen & Roberts 1948). The maximum number of sperms found was 7.35 million and the average was 5.73 million. The distribution was as follows:

Number of Queens	*Sperms (Millions)*
2	3–4
3	4–5
15	5–6
9	6–7
4	7–8

EFFECT OF DRY AIR ON INSEMINATED QUEENS.—In this experiment all queens were inseminated with bulb fluid and sperm from the seminal vesicles of one drone. A mucus plug of 0.2 mm.^{3} was placed in the vagina. The queens were divided into two groups of 11 each. One group was returned to the dry cabinet immediately after insemination, and the other was left in the laboratory, where the relative humidity was higher. The average opacity of the spermathecae of the first group was 5.2 and that of the second group 5.1. The low humidity apparently had no detrimental effect.

COMPARISON OF DRONES RETURNING FROM THE FIELD WITH THOSE MAINTAINED IN DRY CABINETS.—The insemination procedure was the same as above. Sixteen queens were divided into two groups of eight each. One group was inseminated with sperm from drones that had just been caught at the hive entrance as they returned from the field, and the other with sperm from drones col-

lected as they emerged and kept in the dry cabinet 16 to 17 days. The average opacity of the spermathecae of the first group was 5.1 and that of the second group 5.5. These data at least show that drones are not harmed by maintenance in the cabinet.

VALUE OF PENIS-BULB FLUID.—For these inseminations sperm was taken from seminal vesicles, and after injection the vagina was filled with about 0.2 mm.^{3} of mucus. Queens of one group were given 0.5 mm.^{3} of sperm only, those of another group the fluid from one penis bulb and 0.5 mm.^{3} of sperm in one injection, and those of a third group bulb fluid and 0.5 mm.^{3} of sperm from one drone plus the bulb fluid from another drone in one injection. The numbers of sperms (millions) in the spermathecae of these queens were as follows:

	Number of Queens	*Range*	*Average*
Sperm only	11	0.19–0.80	.44
Fluid and sperm	9	0.10–0.68	.40
Fluid-sperm-fluid	11	0.19–0.98	.52

It is not at all clear that bulb fluid has any value. The most sperms reached the spermatheca when fluid from two bulbs was used and the least when fluid from one bulb was used.

VALUE OF MUCUS AS A SEAL.—After natural mating the vagina and posterior part of the reproductive tract of the queen often contain mucus. Some investigators have attached considerable importance to this mucus as a seal to prevent escape of semen, but no data have been presented to prove its value in either natural or artificial mating.

Thirty-two queens were inseminated with 0.4 mm.^{3} of bulb fluid and 0.6 mm.^{3} of sperm. In half these queens the vagina was then filled with mucus. When the spermathecae were compared by the opacity method, the group receiving mucus appeared to be slightly better inseminated.

Queens in another lot were inseminated with semen collected from a single drone by the eversion method. The lot was divided into 3 groups of 10 queens each. In one group the vagina was filled with about 0.2 mm.^{3} of mucus, in another group the vagina opening was smeared with mucus, and in the third group no mucus was used. The summary of the spermathecal counts (millions) given below, shows no advantage in a mucus plug or smear.

	Range	*Average*
Vagina filled	0.64–2.23	1.14
Smear only	0.08–1.90	1.07
No mucus	0.35–2.34	1.34

COMPARISON OF SPERM-COLLECTING METHODS.—In the preceding experiment, in which the eversion method of collecting sperm was used, a greater number of sperms reached the spermatheca than at any time when the sperm was taken from the seminal vesicles. A direct comparison between these sperm-collecting methods was made in the following experiment. Nine queens were inseminated with semen from the completely everted penis of a single drone, and eight with the bulb fluid and sperm from one drone plus the bulb fluid of another drone. Drones were caught at entrances as they returned from the field. The numbers of sperms (millions) in the spermathecae of these queens were as follows:

	Range	*Average*
Complete eversion	0.29–1.05	0.75
Seminal vesicles	0.24–0.83	0.45

The difference between these means is significant at the 2% level. This experiment confirmed the superiority of the eversion method, which was thereafter used exclusively.

EFFECT OF AGE OF QUEEN.—Queens were kept in the low-humidity cabinet, and drones were collected at the hive entrance. Five queens were inseminated each day from the first to the tenth day after emergence. These were single-drone inseminations by the complete eversion method. The numbers of sperms (millions) reaching the spermathecae were as follows:

Age in Days	*Range*	*Average*
1	0.14–0.65	0.43
2	0.28–1.16	0.67
3	0.52–1.28	0.90
4	0.24–1.00	0.69
5	0.44–1.52	0.67
6	0.49–0.97	0.74
7	0.35–0.96	0.54
8	0.44–0.94	0.64
9	0.27–1.09	0.75
10	0.38–1.45	0.91

Apparently age has little effect on insemination success after the first day.

EFFECT OF CARBON DIOXIDE, AIR MIXED WITH SEMEN, AND WATER IN SYRINGE ON SUCCESS OF INSEMINATION.—In this experiment each queen was given 2.5 mm.^{3} of semen taken from the everted penis of several drones. As usual a little air was taken into the syringe to separate semen from the water. Although this air space retarded the mixture of sperm with water, the separation was not complete because some water adhered to the wall of the syringe and mixed with semen as it was drawn in. Carbon dioxide was used during the insemination, which lasted 5 to 10 minutes, and additional 10-minute treatments were given on each of the 2 days following insemination. Queens were maintained in nuclei and drones were confined to a hive. Sperm counts were made soon after the queens started to lay.

The queens were divided into four groups. One group served as control. Another group was placed in the carbon dioxide chamber for 1 hour immediately after insemination. In the third group small amounts of air and semen were taken into the syringe alternately, about one-half as much air as semen. In the fourth group semen of one drone was taken into the syringe first, followed by a small quantity of air and then 2.5 mm.^{3} of semen. Only the last quantity of semen, which had no contact with water, was injected. Eight queens in the control group were inseminated and nine in each of the other groups, but there were some deaths in each group before counts were made.

The numbers of sperms (millions) reaching the spermathecae from each of these treatments are shown below.

Treatment	*Number of Queens*	*Range*	*Average*
Control	7	1.44–4.19	2.89
Queens exposed to carbon dioxide	6	1.33–2.53	2.17
Air mixed with semen	7	1.34–3.73	2.39
No contact of water with semen	8	1.20–3.53	2.35

The average number of sperms reaching the spermatheca was highest in the control group, indicating that the slight mixture of water and semen that takes place in the usual method of insemination is not detrimental. The results also suggest that mixture of air with semen and ex-

posure of queens to carbon dioxide should be reduced to a minimum until more data are available. The differences between the means of the experimental groups and the control are not significant at the 5% level.

EFFECT OF CHLOROFORM.—The queens used in this experiment were divided into two groups of six. The drones used in the insemination of one group were ejaculated and everted by pressure on the thorax and abdomen. Those used in the insemination of the other group were placed in a vial containing chloroform fumes until partial eversion occurred, and then the eversion was completed by pressure on the abdomen. Only one drone was used to inseminate each queen. The summary of spermathecal counts (millions), given below, shows no detrimental effect of chloroform.

	Range	*Average*
Pressure	0.51–2.25	1.12
Chloroform	0.45–1.75	1.29

LARGE AMOUNTS OF SEMEN IN A SINGLE INSEMINATION.—During the spring of 1944 the routine method of insemination in all the breeding work was as follows: Four or five injections of semen were given each queen in one operation, making a total of 10 to 15 mm.^{3}. No anesthetic was used. In one group of 28 queens inseminated 25 started laying. Eleven were given 14 to 15 mm.^{3}, of which 10 started laying. None of the queens were available for sperm counts.

Another group of 27 queens were inseminated in the same way with 10 mm.^{3} of semen 4 to 5 days after emergence. They were anesthetized with carbon dioxide during insemination. Seven queens were lost. A month after emergence sperm counts were made on 13 queens that were still alive and on 7 that had died. Lumps of dead sperm were found in four of the dead queens, suggesting a possible cause of the high death rate. Sperm counts averaged 3.91 million and varied from 1.09 to 6.71 million with a variation coefficient of 40. Mackensen & Roberts (1948) reported an average of 0.87 million and a variation coefficient of 51 when semen from one drone was used, of 2.97 million with a variation coefficient of 35 when 2.5 mm.^{3} of semen was used in a single injection, and of 5.52 million with a variation coefficient of 10 when 10 mm.^{3} of semen was given in four separate inseminations of 2.5 mm.^{3} each. These data give some idea of what can be expected of large inseminations.

ATTAINMENT OF MAXIMUM INSEMINATION.—In the group of 27 queens in which one large insemination of 10 mm.^{3} of semen was given, there were only two sperm counts of 6 million or over (6.0 and 6.7 million).

In 1940 several queens were inseminated with large amounts of semen on successive days, the average amount given at one time being 6.4 mm.^{3}. Sperm counts were made 2 days after the last insemination. One queen given two inseminations had a sperm count of 5.1 million, two queens given three inseminations each had 5.0 and 5.4 million, and two queens given five inseminations had 5.4 and 6.8 million. The average was 5.5 million.

Later 15 queens were each inseminated with 2.5 mm.^{3} of semen on the third, fifth, seventh, and ninth days after emergence. Carbon dioxide was used as an anesthetic, and the queens were killed soon after they started laying. Sperm counts made on the 11 queens that started laying averaged 5.5 million with a range 4.7 to 6.8 million. Only one queen had over 6 million.

In 1952 five queens were inseminated with semen from three drones six times at 2-day intervals between the ages of 4 and 15 days. To prevent oviposition, which might interfere with the later inseminations, no carbon dioxide was used. Sperm counts were made when the queens were 40 days old. The average was 5.2 million, with individual counts 4.6, 4.6, 4.7, 5.9 and 6.0 million.

Of the 33 naturally mated queens 13 contained more than 6 million sperms and 4 more than 7 million. The maximum was 7.35 million, and the average 5.73 million. Although in certain groups of artificially inseminated queens the average number of sperms has nearly reached the average of naturally mated queens, the higher numbers found in naturally mated queens have not been attained.

SUMMARY.—A number of variations in the technique for insemination of queen bees were studied, and observations made on sperm numbers in drones and naturally mated queens.

The number of sperms in the seminal vesicles of caged drones increased with age from 0.13 million at 3–4 days to 9.89 million at 7 to 8 days while hive reared drones contained an average of 10.4 million. Naturally mated queens contained in their spermathecae an average of 5.73 million sperms and a maximum of 7.35 million.

The following did not alter significantly the number of sperms reaching the spermatheca: Exposure of queens to low humidity of 20 to 30 per cent after insemination, addition of penis-bulb fluid to sperm from the vesicles, application of mucus in the vagina after insemination, use of chloroform to ejaculate drones, 1-hour exposure to carbon dioxide after insemination, mixture of air with the semen, and prevention of contact between semen and water in the syringe.

Semen from the everted penis is superior to that from the vesicles. Queens from 2 to 10 days old can be inseminated with equal effectiveness, and those 1 day old with some degree of success.

When large injections of 10–15 mm.^{3} were given in one operation, 3.91 million sperms reached the spermatheca. Greater efficiency was obtained when the same amount of semen was given in several smaller doses. The maximum reached in artificial inseminations was 6.8 million sperms, and very few counts over 6 million were obtained, while in naturally mated queens the maximum was 7.35 million, and 39 per cent contained over 6 million sperms.

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Solutions of Insecticides in an Isoparaffinic Oil for Cotton Insect Control[1]

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The practical use of spray materials for cotton insect control is relatively new. Field tests involving this method of application were begun in 1946 and reports by Stevenson & Kauffman (1948), Watts (1948), Dean & Gaines (1950), Gaines & Wipprecht (1950), Fenton (1950), Chapman *et al.* (1950) and others indicated that organic insecticide sprays could be used as effectively as dusts in combatting cotton insects. In succeeding years the use of sprays increased rapidly, particularly in the western portion of the cotton belt.

Most of the work with sprays for cotton insect control has involved emulsions. Suspensions generally have not been suitable for use in the low-gallonage, weed-type sprayers adopted for the application of cotton insecticides. Emulsions of synthetic organic insecticides, on the other hand, have proven well adapted to the available spray equipment and well suited for dealing with cotton insect problems.

Oil solutions have rarely been considered for cotton. Cost, and the hazard of phytotoxicity have been prohibitive factors. However, oil solutions, if practical, would have some distinct advantages over emulsions. The necessity for having a readily available supply of water for field use is a legitimate argument against spraying on many farms. Getting the proper tank mixture is also of concern to the cotton farmer and control in numerous cases has been unsatisfactory for this reason alone. By eliminating the need for water and care in preparation of the spray mix, ready-to-use oil solution sprays would be as simple to use as dusts.

THE SOLVENT.—An oil known as HFA No. 1[3] or Soltrol 180[3] was reputed to be virtually non-phytotoxic. Its physical properties and lack of phytotoxicity to a number of crop plants have been described by Goodhue (1953).

In the laboratory HFA No. 1 was not injurious to cotton. Tests conducted in cooperation with the Phillips Petroleum Company were initiated to determine (1) whether this oil was sufficiently nonphytotoxic to use on cotton in the field, (2) the degree of phytotoxicity of various insecticides in this oil solution under field conditions and (3) whether such oil solutions would give satisfactory insect control.

All field tests reported here were conducted on the Ross farm near Eufaula, Oklahoma, in McIntosh County using Deltapine 15 cotton.

EXPERIMENTS IN 1951.—Insecticides were applied with a tractor-mounted Meyer sprayer operated by a gear pump from the tractor power take-off. The nozzles used were T-type hollow cone with an orifice of 0.042 inches. Intended rates of application in the field were from 5 to 7.5 gallons per acre. For initial tests, toxaphene, the most widely used cotton spray insecticide in Oklahoma at the time was selected and a formulation containing 2 pounds of toxaphene in 5 gallons of HFA No. 1 was prepared.

On June 25, just as the plants were beginning to square, approximately 0.1 acre was treated. Due to an increased rate of application of the oil solution over the emulsion normally used at the same pressure and tractor speed, 10 gallons per acre rather than the intended 5 were applied. The plants were severely burned. After 2 weeks the plants began to recover and no dead plants were observed. At a later date, plants sprayed with HFA No. 1 without insecticide in solution showed no damage. Because of the indication that it was the toxaphene in oil solution that was responsible for phytotoxicity, the decision was made to substitute aldrin, which could be used at 1 pound in 10 gallons of oil.

Table 1.—Effect on the cotton boll weevil and cotton bollworm of treatment with toxaphene in oil solution[a] and emulsion at Eufaula, Oklahoma, 1951.

Formulation	Per Cent Squares Weevil Infested			Per Cent Bollworm Injury		
				Squares	Bolls	
	July 20	July 30	Aug. 4	July 30	Aug. 4	Aug. 9
Emulsion	7.0	7.5	11.5	0	4.0	5.0
Solution	6.0	10.0	16.0	0.7	7.3	8.7
Untreated	18.0	17.0	17.0	10.0	16.0	15.0

[a] Actually applied as a mixture of HFA No. 1 solution and water and compared with a standard emulsion.

Meanwhile the toxaphene in oil solution was applied mixed with an equal amount of water at the rate of approximately 10 gallons of the mixture per acre. Mixing appeared to be satisfactorily accomplished without the addition of an emulsifying agent, though it is possible that such agent may have been present as a contaminant in the spray equipment. The only agitation was provided by the return of liquid to the spray tank through the overflow hose. This toxaphene-in-oil and water mixture caused only a slight but noticeable foliage "burn" similar to that caused by the conventional toxaphene emulsions.

Aldrin in HFA No. 1 solution was applied in August at the rate of 8 gallons per acre with no noticeable phytotoxic effects. All plots, including the otherwise untreated checks, were treated twice by airplane after ground applications became impractical. Dieldrin-DDT spray emulsion was used for this late protection of the experimental plots. A comparison of the effectiveness of a standard toxaphene emulsion and the toxaphene in oil solution is given in table 1.

Per cent injury by both boll weevils and boll worms ran somewhat higher in plots treated with the oil solution, though not significantly so. Two facts should be pointed out in considering these data. First, where late bollworm control was concerned, aldrin was used after August 7 and this material is relatively ineffective against the bollworm. Secondly, because of the necessity for diluting the solution with water, the rate of application of actual toxaphene was generally lower than that for the emulsion. The

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[3] Trade names of Phillips Petroleum Company, Bartlesville, Oklahoma.

Table 2.—Amounts of insecticide applied to cotton in a comparison of two formulations at Eufaula, Oklahoma, 1951.

Formulation	Rate in Pounds of Toxaphene per Acre					
	June 25	July 3	July 9	July 14	July 25	July 30
Emulsion	2.10	2.52	2.52	2.66	2.66	2.66
Solution	4.00	0.67	1.00	2.00	1.50	2.00

actual amounts of toxaphene applied are shown in table 2.

The yields from plots treated with the two formulations of toxaphene and from the untreated plots were: emulsion, 668 lbs. lint; solution, 707 lbs. lint; and untreated, 462 lbs. lint cotton per acre.

The total area treated with HFA No. 1 oil formulations included in three replicated plots was 1 acre.

Experiments in 1952.—In 1952 attention was turned toward investigating cotton insecticides other than toxaphene and aldrin in HFA No. 1 solutions and in testing the feasibility of using extremely low rates of application of the solutions. Such low rates of application would be necessary if the oil solution were to be practical from an economic standpoint. This necesitated formulation of more concentrated solutions and some difficulty was encountered when certain of the insecticides, particularly DDT, showed a tendency to crystallize and settle out of solution.

Hollow cone T-type nozzles were used, but for this work an orifice diameter of 0.023 inches was selected. To facilitate the application of small amounts of solution, a trailer-mounted sprayer was used and the trailer was equipped to permit the spray solution to be applied from a 5-gallon container. The sprayer was a Yellow Devil operated by a gear pump from the tractor power take-off. The formulations tested were:

0.33 lb. DDT plus 0.13 lb. dieldrin per gallon HFA No. 1
0.33 lb. heptachlor per gallon HFA No. 1
0.166 lb. endrin per gallon HFA No. 1

Rates of application in the field were from 2 to 4 gallons per acre, depending on the size of the cotton plants. The first application with each of these three formulations was made to approximately 0.5 acre. This was on June 23 at which time the cotton had been squaring for about a week. On June 30 a slight burning was noted on cotton treated with the heptachlor solution. Another 0.5 acre plot was treated at this time and the burning was noted again on July 7. However, it was less severe than that caused by a standard chlordane emulsion and it was decided to proceed with treatment of all plots as planned. The young plants which suffered moderate foliage injury recovered, and as they grew older they showed less injury when sprayed. No phytotoxic effects were evident due to the use of the dieldrin-DDT or endrin formulations.

In this test the various formulations in oil solutions were compared only with an untreated check and not with equivalent emulsions. Their relative effectiveness against the principal cotton pests is shown in table 3. The yields from plots treated with these formulations and from untreated plots were: dieldrin-DDT, 532 lbs. lint; endrin, 360 lbs. lint; heptachlor, 259 lbs. lint; and untreated, 144 lbs. lint cotton per acre.

Cotton was grown under less favorable conditions during 1952 than in 1951 and the experimental plots did not receive the late aerial protection. Each treatment was replicated four times involving total acreage as follows: dieldrin-DDT, 3.0 acres; endrin, 2.8 acres; and heptachlor, 2.8 acres.

Summary.—The oil known as HFA No. 1 or Soltrol 180 is non-phytotoxic to cotton when applied in the field at rates up to 7.5 gallons per acre. Insofar as cotton insect control was concerned, no decrease in effectiveness of the insecticides tested was evident when they were applied in a solution of this oil. Rates of application for oil solutions of 2 to 4 gallons per acre appeared to be effective on cotton under Oklahoma conditions.

While the oil itself proved non-phytotoxic, solutions of the various insecticides in the oil had varying detrimental effects on the plants. Toxaphene in HFA No. 1 solution was highly injurious to cotton plants at rates normally applied under field conditions. Heptachlor in the oil solution caused moderate injury to young cotton plants from which the plants were able to recover. Older plants showed less injury when sprayed at practical field rates.

Aldrin, dieldrin plus DDT, and endrin in HFA No. 1 solutions were nonphytotoxic to cotton at practical rates of application.

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Table 3.—Injury by cotton insects to cotton treated with solutions of insecticides in HFA No. 1 oil at Eufaula, Oklahoma, 1952.

Formulation	Per Cent Squares Punctured by Boll Weevil				Per Cent Squares Injured by Bollworms			Per Cent Bolls Injured by Bollworms Aug. 11
	Max.	Min.	Ave. 5 Counts	Ave. 7 Counts	Max.	Min.	Ave. 3 Counts	
Dieldrin-DDT	11.0	2.5	8.4	7.6	8.0	1.5	3.8	6.0
Endrin	16.0	3.5	8.0	7.9	10.0	1.0	4.3	9.0
Heptachlor	24.5	8.0	12.0	—	12.0	4.5	7.7	19.0
Untreated	30.0	11.3	21.8	19.5	32.5	5.0	19.0	42.5

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Status of Analytical Methods with Respect to the Determination of Minimal Quantities of Insecticides[1]

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The increased use of insecticides on agricultural crops, soil, and livestock has made it necessary to develop methods for determining residues of these materials in biological products of all kinds. Fresh and frozen fruits and vegetables, canned goods, stored products, cereal and forage crops, and animal products such as poultry, milk, butter, and meat—all have to be examined for the presence of extraneous chemicals.

Residues on and in field crops may result from the direct application of insecticides to the growing plants or from the uptake of the chemicals from the soil. Animal products may contain residues as a result of application to the animal in spraying and dipping operations or from feeds containing them. Stored products may be contaminated from treatments for their protection against insect attack during storage. Soils may become contaminated from the direct application of insecticides or from applications to crops over a period of years.

The older insecticides include the arsenicals, fluorine compounds, sulfur, oils, plant materials such as pyrethrum, derris and cube, nicotine, and ryania. The newer insecticides can be generally classified into three categories—compounds containing organically bound chlorine, the organic phosphorus compounds, and some nitrated compounds.

Proposed tolerances recently announced by the Food and Drug Administration (1954) specify amounts of pesticide chemicals that may be present on fresh fruits and vegetables, ranging from zero for some materials up to 14 p.p.m. for methoxychlor. Sulfur, some copper compounds, pyrethrum, rotenone, ryania, sabadilla, and some synergists were considered to be safe when used in accordance with good agricultural practices, and were exempted from the requirements of a tolerance. For many of the pesticides now in use sufficient data were not available for establishing a tolerance.

Accuracy and precision in the methods of analysis are of prime importance in determining the deposits from pesticide applications and the residues that may be present at harvesttime.

SAMPLING PROCEDURES.—Before chemical analyses are made several factors must be considered. The sampling procedures must be adequate to secure representative samples. Samples may be selected with two objectives—to check the uniformity of application, and to determine the average residue on the commodity. To check uniformity of application a number of samples are selected and analyzed as individual units. In most of our forage-crop experiments all the vegetation on each of five 1-square-yard areas was treated as a separate unit. Composite samples representing the entire area were obtained by combining a number of small samples and subsampling them. The same general principles apply to sampling other crops.

The size of the sample depends on the crop and kind of material. For fresh fruits and vegetables, green vegetation, and similar products 1-kg. samples are convenient to handle in the laboratory and generally contain sufficient toxicant for reliable determinations at the tolerance level. For instance, a 1-kg. sample of cabbage would contain 7 mg. of toxaphene at the tolerance level of 7 p.p.m., and this amount can be readily determined. For determining residues at the 0.1 p.p.m. level, refinements in the procedures and more precise methods are desirable.

PREPARATION OF SAMPLE.—After selection of the sample the next step is a preliminary separation of the toxicant from the large mass of plant or animal material with which it is associated. For instance, in the determination of surface residues of DDT on apples, they are put in a large container, a measured amount of an organic solvent such as benzene is added, and the container is rolled or agitated to bring the solvent into contact with the residue, which is thus stripped from the surface. This procedure is useful for fruits, vegetables, forage crops, and other materials that will withstand rolling or tumbling. For soft fruits, berries, and like materials extraction in a Soxhlet apparatus, or simply by soaking in an organic solvent, is practical. In general, the organic-solvent phase containing the toxicant will separate from the aqueous phase and the large mass of plant materials.

Peeling of fruits and vegetables followed by drying and extraction with a solvent is sometimes resorted to. When it is desired to determine the toxicant within the material, as in potatoes and other root crops, it is necessary to grind or macerate the material before extraction. An ordinary food chopper or Waring Blendor is useful for this operation.

The extract, or strip solution, of plant materials, especially of oil-bearing plants such as peanuts, corn, and soybeans, always contains oils, waxes, and coloring matter. Before colorimetric determinations or bioassays of such extracts can be made it is necessary to subject the solutions to clean-up procedures. Some clean-up procedures are useful in the preparation of samples for organic-chlorine determinations.

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There are a number of clean-up procedures in common use; the choice depends on the nature of the toxicant and the material with which it is associated. Extracts containing DDT and glycerides can be cleaned up with fuming sulfuric acid. This treatment sulfonates the oils and waxes, rendering them water-soluble without affecting the DDT, which can be extracted from the mixture with chloroform. Several of the insecticide materials will withstand this treatment; others will not. Extracts containing dieldrin or aldrin associated with glycerides may be treated with alcoholic potash, which saponifies the organic acids, rendering them water-soluble without affecting the toxicant.

Chromatographic procedures are often very useful for the separation of the toxicant from associated material. These procedures consist in filtering the strip solution through a column of adsorbent material such as silicic acid, aluminum or magnesium oxide, charcoal, attapulgus clay, and various combinations. Mixtures of immiscible solvents such as normal hexane and nitromethane are used as carriers and solvents. The plant material is held on the column while the toxicant goes through, or vice versa. No single adsorbent and set of solvents are applicable to all separations. Each toxicant and each biological material is susceptible to different treatments.

Insecticides that are volatile with steam can be separated by steam distillation.

METHODS OF ANALYSES.—After the strip solutions have been prepared, and subjected to clean-up procedures if necessary, quantitative chemical analyses are in order. Specific colorimetric and spectrophotomertic determinations are available for some of the toxicants; for others only general methods have been developed.

One method commonly used for the chlorinated hydrocarbons is the determination of the organic-chlorine content of the strip solution. This is a quick screening test for the presence of chlorinated hydrocarbon pesticides in biological materials. It may be carried out in two ways. In one way the biological extract is concentrated down to a small volume and then refluxed for 2 hours or more in isopropyl alcohol plus metallic sodium. In another procedure the concentrated extracts are burned in a combustion furnance, and the products of combustion are absorbed in a solution of sodium carbonate. By both procedures the organically bound chlorine is converted to ionic chloride.

Two types of combustion furnace are available. One consists of quartz tubes electrically heated and arranged horizontally. The strip solution is concentrated down to a small volume in quartz capsules, which are then inserted into the furnace. Another type of furnace consists of quartz tubes electrically heated and arranged vertically. In this furnace the sample must be a liquid or in a solution, which is allowed to drop slowly into the combustion chamber. In both types of furnace air or oxygen is drawn through and the products of combustion are absorbed in a sodium carbonate solution.

The chlorine liberated by these procedures may then be determined by electrometric or amperometric titration or other standard procedures. By use of special precautions, 10 micrograms and less of chlorine can be determined. This is 0.01 p.p.m. on a 1-kg. sample or 0.1 p.p.m. on a 100-gram sample.

Organic-chlorine determinations are not specific, and are always run in comparison with similar determinations on untreated materials. Naturally occurring inorganic chlorides in plants are not soluble in the organic solvents used for stripping and therefore cause a minimum of interference.

Specific colorimetric and spectrophotometric methods have been developed for DDT, BHC, chlordane, methoxychlor, aldrin, dieldrin, endrin, heptachlor, parathion, malathion, but homologous compounds, decomposition products, and plant extractives sometimes cause interference. Generally these methods are tedious and time-consuming. Many of these colorimetric methods are accurate for the determination of 1 to 150 microns, so that it is sometimes possible to detect residues considerably less than 0.1 p.p.m.

For determining residues of the inorganic insecticides we have accurate and precise methods for arsenic, lead, fluorine, copper, antimony, and some other elements. These methods are recognized as a basis for calculation of the insecticide content of the material.

For residues of the organic-phosphorus compounds an enzymatic method is sometimes used. This method is based on the inhibition of cholinesterase activity but is not specific. It is sensitive to very small amounts, so that residues less than 0.1 p.p.m. can be determined.

Bioassay procedures are also useful in confirming the presence of toxic residues in plant and animal products, although they are not specific. Bioassays have sometimes indicated the presence of toxic residues that had not been detected by chemical analysis.

CONCLUSIONS.—In conclusion, it is feasible to determine residues of most of the insecticides now used on agricultural crops at tolerance levels that have been announced. Complete reliance should not be placed on any one method, but a combination of two or more, perhaps including bioassay, should be used. It has been found that certain compounds when ingested by animals are metabolized into other compounds which do not react to the specific test for the original compound. Some of the systemic organic-phosphorus compounds are apparently metabolized by plants into compounds that are more toxic than the original.

REFERENCE CITED

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Oviposition Preference of *Meromyza americana* Fitch for Different Small Grain Varieties under Greenhouse Conditions[1]

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The wheat stem maggot, *Meromyza americana* Fitch (Diptera; Chloropidae) is a well known and widely distributed insect pest of North America which injures wheat, barley, rye and grasses. Gilberston (1925) gave a good description of the life history and the injury produced. He as well as Dunham (1934) reported evidence of resistance in wheat varieties to injury from the wheat stem maggot. Allen & Painter (1937) reviewed the literature up to that date, consisting mainly from short notes in annual reports and technical papers which dealt with the life history, parasites and food plants. Furthermore, they reported the results of an extensive study on differential infestation of wheat varieties. The relation of stage of plant maturity to the number of blasted heads was found to be an important characteristic for several wheat varieties. The studies reported in this paper were mainly directed toward bringing together more evidence on the behavior of the fly on wheat varieties before and during oviposition.

METHODS.—Oviposition tests were carried out in flats each permitting the planting of about 300 seedlings. Several flats in the same experiment were covered with a cheesecloth tent and exposed to the same batch of ovipositing flies. The tents were illuminated with four daylight type G. E. fluorescent lights, thus reducing greatly any etiolation of the plants. These were grown at the temperatures ranging between 20° to 30° C. which were favorable for oviposition. Volunteer wheat brought in from October to early May proved to be an abundant source of wheat stem maggots. Greenbugs, *Sciara* larvae, seed-corn beetles and parasitic wasps interfered somewhat with the maintenance of large populations of flies in the greenhouse.

Table 1.—Preference of *Meromyza americana* shown by oviposition on different small grain varieties in the greenhouse.

VARIETIES TESTED	AVERAGE NUMBER OF EGGS PER PLANT (ANGLES)
Comanche wheat	5.33
Cheyenne wheat	5.05
Ponca wheat (test rows)	4.50
Ponca wheat (border rows)	4.61
Wichita wheat	4.29
Tenmarq wheat	4.17
Blackhull wheat	4.12
Balbo rye	4.10
Turkey wheat	4.09
Pawnee wheat	3.76
Harvest Queen wheat	3.19
Red Chief wheat	2.89
Dictoo barley	1.65
Reno barley	0.57
Cherokee oats	0
Kanota oats	0
Mo. 0-205 oats	0
Average	3.09
L.S.D. 0.05	1.66
0.01	2.24

Table 2.—Preference of *Meromyza americana* as shown by oviposition on six wheat varieties in the greenhouse.

VARIETY	AVERAGE NUMBER OF EGGS PER 100 PLANTS (ANGLES)	
	Second Test	Third Test
Ponca	39.13	39.03
Comanche	37.91	38.08
Red Chief	33.73	26.25
Cheyenne	30.99	27.41
Pawnee	30.24	25.54
Harvest Queen	8.10	15.59
Average	30.01	28.65
L.S.D. 0.05	11.81	11.29
0.01	15.80	15.40

In the first test, the wheat varieties Blackhull, Cheyenne, Comanche, Harvest Queen, Pawnee, Ponca, Red Chief, Tenmarq, Turkey, Wichita, the rye variety Balbo, the barley varieties Dictoo and Reno and the oat varieties Cherokee, Kanota and Mo. 0–205 were planted on December 31, 1952. Each of the three flats had a single row with about 10 plants of each variety. Soon after emergence of the coleoptile, ovipositing flies were released in the cheesecloth tent. Counts were made on January 10, 17, and 24. The range of preference during oviposition shown in table 1 is based on the final count. Three weeks after the first oviposition the first evidence of plant damage was detected.

The second and third oviposition test included the following six wheat varieties: Cheyenne, Comanche, Harvest Queen, Pawnee, Ponca and Red Chief. The second test was planted in 9 blocks with each of 6 different varieties in a single row of 10 to 12 plants. The third test included these varieties in 3 blocks each containing 3 adjacent rows of the same variety. Each test was planted in two flats. All four flats were covered by the same tent. The eggs were counted on February 15, 23, March 8 and 23. The results given in table 2 are based on the records of the final count.

The fourth test was set up to give more evidence on the influence of the vigor of the plants on the preference during oviposition since in the earlier tests it appeared that bigger plants sometimes were preferred to smaller ones. For this reason, each of the same 6 varieties of the previous experiments were sown at 3 different dates at an interval of 10 days and exposed to the flies as soon as

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Table 3.—Preference during oviposition showing interaction between three seeding dates with six wheat varieties.

VARIETY	AVERAGE NUMBER OF EGGS PER 100 PLANTS (ANGLES)		
Pawnee	41.26	1[a]	49.81
		2	46.83
		3	27.15
Comanche	40.03	1	36.66
		2	44.03
		3	39.40
Ponca	39.57	1	51.63
		2	35.88
		3	31.21
Cheyenne	34.65	1	43.84
		2	32.95
		3	27.10
Harvest Queen	22.39	1	20.11
		2	29.13
		3	17.95
Red Chief	17.79	1	17.59
		2	15.14
		3	20.67
Average	32.62		
L.S.D. 0.05	10.77		
0.01	14.69		

[a] Seeding dates.

the last sown kernels germinated. Each variety was sown in 3 blocks at 3 different dates, with 2 rows side by side consisting of about 10 plants in each row. The results given in table 3 are based on the final count.

The objective of the fifth test was to get more evidence on the influence of the color of the seedlings during oviposition. For this purpose, the flats containing the same 6 wheat varieties in 4 blocks with a single row of each variety were exposed to white, yellow, red or blue light. Each flat was isolated during oviposition in a cardboard box covered with a sheet of transparent colorless, yellow, red or blue cellophane. Two sets of fluorescent daylight tubes served as light sources. The introduced flies had no choice between the different compartments or colors, but they had the liberty to choose one or another of the grain varieties of the same flat. Since oviposition was poor under red light and the least under blue light, more flies were released in these compartments and the experiments repeated. The results based on the final counts are given in table 4. Since the different flats were not exposed to the same group and number of flies the results of each test were compared in a separate analysis of variance. According to a suggestion of Snedecor (1950), the analysis of variance was run twice for all tests, first with the original data obtained as percentages and then with transformed values e.g. percentages converted into angles (angle = arc sine $\sqrt{\text{percentage}}$). In the tables 1 to 4, only the latter computation is given. Only minor differences were obtained by calculating the means and the least significant differences by the two different methods. They did not influence the conclusions.

RESULTS.—*Oviposition on Wheat, Rye, Barley and Oats.*—In the first test for preference during oviposition of *M. americana*, there was a significant difference between the oats on the one hand on which no eggs were laid (with one single exception) and the wheat on the other upon which most of the eggs were laid (Table 1). The 2 barley varieties showed only a few eggs and were significantly less preferred than 9 of 11 wheat varieties. Balbo rye ranged in the middle ranks of preference comparable to the wheat varieties Wichita, Tenmarq, Blackhull, Turkey and Pawnee which were highly significantly preferred to the 2 barley and the 3 oat varieties. Among the 11 wheat varieties, Comanche was highly significantly more attractive than Red Chief. Furthermore, Comanche was significantly more attractive than Harvest Queen. Cheyenne was significantly preferred to Harvest Queen and Red Chief. Ponca, estimated independently in the border rows as well as in the regular rows within the flats, belongs to the more preferred group after Comanche and Cheyenne being just significantly different from Red Chief. Blackhull had only a few more eggs than Pawnee, Harvest Queen and Red Chief. Wichita and Tenmarq belong to the intermediate group since no particular preference was shown for these varieties. Red Chief and Harvest Queen were the least preferred varieties being significantly less attractive than Comanche and Cheyenne.

Usually only one egg was laid on the same tiller of a plant. However, Comanche, Cheyenne and Ponca had the most plants on which more than one egg was laid,

Table 4.—Oviposition by *Meromyza americana* on six different wheat varieties under different colors of light in greenhouse.[a]

CLEAR CELLOPHANE		YELLOW CELLOPHANE		RED CELLOPHANE				BLUE CELLOPHANE			
				I. Experiment		II. Experiment		I. Experiment		II. Experiment	
Po.[b]	29.45	Po.	32.22	Co.	39.44	Po.	18.84	Ch.	37.40	Ch.	30.29
Pa.	16.48	Co.	20.14	Po.	31.75	Ch.	18.28	R.C.	35.56	Po.	25.60
Co.	11.58	R.C.	17.34	Pa.	31.55	R.C.	9.32	Po.	23.55	R.C.	19.55
Ch.	4.84	Ch.	15.76	R.C.	28.82	Co.	4.61	*H.Q.*	*16.49*	*H.Q.*	*16.24*
R.C.	0	*H.Q.*	*13.61*	*H.Q.*	*14.14*	*H.Q.*	*3.84*	Pa.	14.26	Pa.	14.26
H.Q.	*0*	Pa.	11.12	Ch.	11.37	Pa.	3.55	Co.	4.84	Co.	10.47
Av:	10.39		18.36		26.18		9.74		22.02		19.40
L.S.D. 0.05:	18.28		20.80		17.10		17.48		17.59		25.10
0.01:	25.28		28.76		23.64		24.18		24.32		34.72

[a] Expressed as number of plants infested by eggs per 100 plants; statistical analysis by angle transformation.
[b] Po., Ponca; Pa., Pawnee; Co., Comanche; Ch., Cheyenne; R.C., Red Chief; H.Q., Harvest Queen.

demonstrating again the greater preference over the other varieties. On the latter, none or but a few plants had more than one egg each.

Oviposition on Six Wheat Varieties.—In the second test, Harvest Queen again was significantly less preferred than any other wheat variety in the experiment (Table 2). Ponca and Comanche again had the highest number of eggs whereas Red Chief, Cheyenne and Pawnee ranged in a medium position.

In the third test, Harvest Queen again was the least preferred variety for oviposition with highly significantly fewer eggs than Ponca, Comanche and Cheyenne (Table 3). Cheyenne leads an intermediate group with Red Chief and Pawnee. Ponca and Comanche were the most attractive varieties in the test.

Influence of the Vigor of the Wheat Seedlings on Oviposition Preference.—In the fourth test, Harvest Queen again was among the two least preferred varieties, being highly significantly different from Ponca, Comanche and Pawnee (Table 3). Red Chief in this test was highly significantly less preferred than Cheyenne, Ponca, Comanche and Pawnee. Cheyenne was in an intermediate position, whereas Pawnee was the most preferred variety in addition to Comanche and Ponca.

In addition to the differences between the varieties, there were significant disparities in oviposition between the three succeeding seeding dates of the same variety. The first seeding dates of Pawnee, Ponca and Cheyenne had the most, and the last dates the least number of eggs. The differences in Pawnee were highly significant between the first two dates and the last one, whereas in the varieties Cheyenne and Ponca the differences were significant between the first and the two following counts, but highly significant between the first and the last date. In Comanche and Harvest Queen the seedlings of the second date were preferred, whereas in Red Chief the plants of the last date had the most eggs. In Harvest Queen, the difference between the second and third dates was significant, whereas the other preferences were too small to be significant.

Influence of the Color of the Wheat Seedlings on the Preference for Oviposition.—In the fifth test the rank of preference for oviposition of most of the six varieties of winter wheat was changed under different colors of light (Table 4). Ponca generally belonged to the preferred wheats except under blue light and in one experiment also under red light. Pawnee which was most preferred in white light became the least preferred in yellow and in one experiment with red. Comanche took a varying position from the most preferred in an experiment with red to an intermediate position in the other experiment with red as well as in yellow light. It was the least preferred wheat under blue light. Cheyenne, taking an intermediate place in white and yellow light, was the most preferred wheat in blue, whereas in red it changed in rank from highly preferred to least preferred. Red Chief belonged to the least preferred wheats in white light and in the other colors did not deviate from a median rank except in an experiment with blue light, where it received a rather high number of eggs. Harvest Queen again took the most stable position belonging in all tested colors among the three least preferred varieties, never quite reaching the average oviposition of the tests. The results indicated an interference between the range of wavelength reflected from the plants and the preference for certain varieties. Presumably Harvest Queen did not reflect the attractive wavelength in the tested colors as did some of the other varieties.

DISCUSSION.—As repeatedly demonstrated in these five tests the differential infestation of wheat seedlings of six varieties by the wheat stem maggot can be explained by preference during oviposition. The same conclusions were reached by Cunliffe (1937) and by Cunliffe & Hodges (1946) in their extensive studies with the frit fly (Diptera-Chloropidae).

Orientation of the adult *Meromyza americana* as it was observed in the oviposition tests was influenced by the stage of growth of the seedlings or a factor closely associated to it, and by the intensity of certain wavelengths of the light reflected by the plants. However, neither of the two factors explains the observed preference alone for all varieties exposed in the experiments.

Probably additional factors are involved in oviposition preference. The several factors interfering with preference may provide stimuli during orientation of the fly when moving to the plants as well as immediately before or during oviposition while staying on the plant seeking the most suitable place for oviposition. Individual characters in the behavior of the flies or the exterior of the plants may often modify temporarily or systematically the preference during oviposition.

In addition, environmental influences such as the presence of other flies or eggs, aphids, honeydew or guttation water on some plants may also condition the stimuli responsible for preference. The several stimuli involved may act upon the ovipositing fly together at the same time or consecutively.

Brightness of illumination together with optimal temperatures may have induced the mature female to start or continue with maximal oviposition. Consecutively, only the presence of plants reflecting the preferred wavelength attracting them to the emitting plants and the presence of seedlings of the preferred growth stage made oviposition finally possible. Then a new chain of stimuli may have started. The presence of preferred and less preferred plants together in such close range as in flats or pots in the greenhouse may have veiled the true behavior of the fly under field conditions. That may be explained by the usual situation in which the chain of stimuli is once advanced to a certain step in which oviposition takes place even though final stimuli of the preferred range are lacking or not fully congruent with those provided by the attractive host plant individuals.

Eggs were frequently observed on glass, toothpicks, hairy crab grass, which are quite different from a wheat seedling. If this is true, non-preferred plant individuals should get more eggs when surrounded by specimens of preferred varieties, whereas preferred plants hidden among non-preferred should get less. By inspecting the oviposition flats after pulling out the plants in which the arrangements of toothpicks indicated the plants with eggs, it was observed that the least preferred Harvest Queen got more eggs in a block where by chance all the neighboring rows were preferred varieties. Moreover, in

the third and fourth oviposition test the analysis of variance gave evidence that such a relationship occurs since besides the variance between varieties, that between blocks and furthermore variance caused by interactions between blocks and varieties as well as that between blocks and rows were of considerable size.

As to the choice between the three adjacent rows of the same variety, there was slightly higher oviposition in the center row of the preferred varieties Ponca, Comanche, Cheyenne, Pawnee and Red Chief, approaching the expectation that in attractive varieties the middle rows and in non-preferred varieties, the border rows would receive more eggs. Cunliffe & Hodges (1946) in their studies of the frit fly, which also prefers the coleoptile for oviposition, observed that those flies were placing more eggs in the barley coleoptile than on that of oats. The authors connected that observation with the earlier separation of the coleoptile of barley.

Effects of hairiness or roughness of the surface have not been investigated in the case of *M. americana* since eggs laid on glass were frequently observed as well as oviposition on the rough surface of the soil or on toothpicks or hairy leaves of crab grass. Furthermore, chemotactic or olfactometric measurements with *M. americana* were not investigated.

It is possible that the use of fertilizers may affect the mechanism of preference during oviposition by *M. americana* through growth and color of the seedlings. Nowicki (1871), Schnauer (1929), Blunck & Munkelt (1926), Chrzanowski (1926), and Watzl (1931) observed that there was such a relationship in resistance to the gout fly, *Chlorops pumilionis* Bjerk. Wheat varieties with rapid growth may escape an attack. These observations were confirmed by fertilization experiments with which growth rate in the same variety was influenced. According to Frank (1900), Frew (1924), Kopp (1926), Sappok (1927) and Willimsky (1926), the infestation by the gout fly was increased by a high top nitrogen fertilization in the spring. Frew (1924) and Mathetes (1924) observed both decrease and increase in infestation of barley depending on the fertilizer mixture applied. Horber (1950) concluded from his fertilization experiments on wheat and barley that the application of those nutrients which are in a minimum in the soil may reduce the infestation by the gout fly. Some of these experiments should be repeated with careful consideration of the correlation between growth rate and color of the plants during oviposition and the infestation rate.

The presence of a factor governing infestation by wheat stem maggot which is connected with the stage of growth on wheat varieties was suggested by Allen & Painter (1937). Plants from late sowings were more heavily infested in the spring than plants from earlier sowings. This observation may be explained by oviposition preference. The earlier seeding date was in the proper stage to attract the flies when no adults were present because low temperatures retarded the development of the flies, whereas the later sowings were in the preferred stage at the time when *M. americana* was emerging and ovipositing. The observation that an advanced stage of growth may protect the plants from oviposition by frit fly is widely used by farmers in Europe by sowing the spring varieties of oats, barley and wheat as early as possible.

SUMMARY.—Differences in infestation of various small grains, especially wheat varieties by the wheat stem maggot under greenhouse conditions were demonstrated to be caused by preference shown during oviposition. Color of the light reflected from the plants and the growth stage of the seedlings influence the attraction of the flies to a preferred host variety. Presumably, still other factors conditioning attractiveness are involved. The relation between preference during oviposition and the earlier observed influence of different seeding dates as well as that which may be exerted by the use of fertilizers on the infestation rate are discussed.

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The Toxicology of Homologs and Derivatives of Bayer L 13/59[1]

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We have seen no report in the literature indicating that insects are able to acquire sufficient resistance to prevent the effective use of the organic phosphorus insecticides such as parathion. For this reason, and also because of the great toxicity of many of these materials to higher animals, at a number of laboratories a search has been made for related chemicals that will retain high insect toxicity but be much less toxic to warm-blooded animals. As a result of such research 0,0-dimethyl-2,2,2-trichloro-1-hydroxyethyl-phosphonate, commonly called Bayer L 13/59, has been introduced as an insecticide.

Recently Mattson *et al.* (1954) dehydrochlorinated L 13/59 by the use of alkali and found that the product was more toxic to insects than the parent compound. Barthel *et al.* (1954, 1955) have synthesized the ethyl, propyl, isopropyl, and butyl homologs of L 13/59, and in addition have prepared the dehydrochlorination product of L 13/59, dimethyl-2,2-dichlorovinyl phosphate, and its ethyl, propyl, and isopropyl homologs. At the Beltsville, Md., laboratory we have determined the toxicity of these compounds to house flies and the inhibitory effect on cockroach cholinesterase.

In the toxicity tests individual house flies, *Musca domestica* L., laboratory-reared on standard Peet-Grady medium (Anonymous 1951), were treated topically with the desired amount of the material contained in 1.213 microliters of an acetone solution by means of the device described by Roan & Maeda (1953). Twenty or 40 female flies 3 to 4 days old were used for each test. Two strains were used, insecticide-susceptible and insecticide-resistant. The susceptible strain was derived from the 1948 NAIDM strain and the resistant insects were from the Orlando-Beltsville strain, which has been selected for DDT resistance for more than 150 generations. Preliminary tests were run to determine dosages that would straddle the 50 per cent mortality level. The 24-hour mortality data were plotted on log-probability paper and LD-50's read. Since precise estimates were not required for this study, the data were not subjected to statistical analysis. However, to determine whether pure and technical samples of L 13/59 differ significantly in toxicity, the mortality data for these materials were subjected to probit analysis as described by Finney (1952).

For more than 5 years we used house fly heads as a source of very active cholinesterase, and the technique used to determine the inhibitory effect of various substances was considered almost foolproof. Recently erratic results were obtained, and it was found that brei preparations lost their activity rapidly, the activity being negligible within 30 minutes after preparation instead of lasting for many days as in the past. Salts used in the preparation of the buffers were taken from unopened bottles, the pH and types of buffers were changed, the acetylcholine bromide used for substrate was recrystallized, all glassware was especially cleaned, different techniques were used for grinding, and the flies were immobilized by use of cold instead of carbon dioxide as was done formerly, but the activity still disappeared. Other strains from different laboratories were tested, with similar results. It is, of course, possible that the phenomenon is real instead of an artifact, but we are not yet ready to so accept it. The difficulty is specific for house flies, as roach head, honey bee head, and cattle red blood cell preparations run parallel in alternate glassware behaved normally and retained activity as in the past. Because of this difficulty American cockroaches were used as the source of the enzyme. This enzyme differs somewhat from that found in house fly heads (Babers & Pratt 1951).

For the determination of enzyme activity a brei was prepared by grinding the heads of American cockroaches of mixed sexes in the buffer used by Chadwick & Hill (1947), one head to 2 ml. of buffer. To 0.8 ml. of this brei was added 0.1 ml. of acetone solution of the desired inhibitor and the mixture incubated for 30 minutes at 37.5° C. Then 0.1 ml. of standard acetylcholine bromide solution was added and the mixture again incubated for 30 minutes. At this time the amount of acetylcholine hydrolyzed was determined by Hestrin's (1949) method. The results were plotted, and the molar solution required for 50 per cent inhibition (IN-50) was read from the graph. The results of these and the toxicity tests are shown in table 1. The relative toxicity and inhibitory effects were obtained from the inverse ratios of the LD-50's and IN-50's, each compound being compared with technical L 13/59. From examination of the errors for technical and pure L 13/59, it is evident that the apparent differences in relative toxicity are not significant with either the normal or resistant flies.

Because the homologs of L 13/59 thus far tested against susceptible flies showed so little toxicity, tests were not

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Table 1.—**LD-50's for susceptible and resistant female house flies and the molar concentrations required to cause 50 per cent inhibition of cockroach cholinesterase for homologs and derivatives of Bayer L 13/59.**

Compound	LD-50				IN-50	
	Susceptible Flies		Resistant Flies			
	Micrograms per Fly	Ratio to Technical L 13/59	Micrograms per Fly	Ratio to Technical L 13/59	Molar Concentration	Ratio to Technical L 13/59
L 13/59:						
Technical	0.395±0.038	1	0.663±0.067	1	6×10^{-5}	1.0
Purified	0.315±0.008	1.3	0.601±0.038	1.1	4.2×10^{-5}	1.4
Ethyl homolog	>5.0	—	—	—	6×10^{-4}	0.1
Propyl homolog	>5.0	—	—	—	$>10^{-3}$	<0.01
Isopropyl homolog	>5.0	—	—	—	$>10^{-3}$	<0.01
Butyl homolog	>5.0	—	—	—	$>10^{-3}$	<0.01
Dehydrochlorinated compounds:						
Dimethyl-2,2-dichlorovinyl phosphate	0.013	30.0	0.037	17.9	9.4×10^{7}	64.0
Ethyl homolog	0.031	12.7	0.19	3.5	5×10^{-7}	120.0
Propyl homolog	0.27	1.5	0.81	0.8	1.7×10^{-4}	35.2
Isopropyl homolog	0.30	1.3	0.91	0.7	8×10^{-4}	7.5

made with all compounds on insects from the resistant strain. Although this strain had never been exposed to any insecticide but DDT, some resistance to the several materials having appreciable insecticidal action was apparent. It was also apparent that lengthening the carbon chains connected by oxygen to the phosphorus atom greatly reduced toxicity, much more so in the case of the L 13/59 homologs than with the dehydrochlorinated compounds.

The data show rather close inverse correlation between toxicity and the amount required to inhibit 50 per cent of the cholinesterase activity. It is possible that the reversals noted may disappear when we are again able to use the esterase from house fly heads.

It has been well established that resistant flies are more able to dehydrochlorinate DDT than are susceptible insects, and Perry & Hoskins (1951) have found that the quantity of dehydrochlorinating enzyme present is a good measure of resistance. Sternburg and his collaborators (1950, 1953) attribute the development of resistance to the ability of the insect to synthesize the enzyme. In the case of DDT the product of dehydrochlorination is the rather inoccuous compound DDE. In the case of Bayer L 13/59, the dehydrochlorination product is 30 times as toxic to house flies as the parent compound. Because related groupings are apparently involved in the dehydrochlorination of both insecticides, it will be interesting to learn if L 13/59 can be degraded by the enzyme isolated in such large quantities from resistant house flies.

SUMMARY.—The amount of topically applied toxicant required to cause 50 per cent mortality of female susceptible and resistant house flies, *Musca domestica* L., was determined for Bayer L 13/59 and its ethyl, propyl, isopropyl, and butyl homologs and for the dehydrochlorination products of all but the butyl homolog. The molar concentration of each material required to cause 50 per cent inhibition of cockroach cholinesterase was also determined. The toxicity and cholinesterase inhibitory action of the dehydrochlorinated products were much greater than for L 13/59 but generally decreased with lengthening of the carbon chain connected by oxygen to the phosphorus atom. The resistant strain was somewhat resistant to all materials tested.

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Factors Contributing to the Natural Control of Citrus Insects and Mites in Florida[1]

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Injurious insects and mites cause large annual losses of the citrus fruit crop in Florida. These losses, although expressed in reduction in yield, are a reflection of leaf, fruit, twig, and root injury by many minor and a few major injurious species. The losses would be even greater were it not for a relatively high degree of natural control which prevents many of the minor species from becoming of economic importance and to some extent limits injurious populations of the major species. At the present time biologic agents such as predators, insect parasites, and pathogenic agents appear to be the most important factors in the natural control of citrus insects and mites in Florida. This paper is concerned with a survey of these factors on the major injurious insects and mites and a preliminary evaluation of their importance to citrus production in the state.

The survey of pathogenic agents, parasites, and predators, revealed a total of two pathogens, 11 insect parasites, and 14 predators attacking the two major scale insects, purple scale. *Lepidosaphes beckii* (Newm.), and Florida red scale, *Chrysomphalus aonidum* (L.). The three major injurious mites, the citrus rust mite, *Phyllocoptruta oleivora* (Ashm.), the citrus red mite, *Metatetranychus citri* (McG.), and the six-spotted mite, *Eotetranychus sexmaculatus* (Riley), were found to be attacked by four pathogens and 11 predators.

Evaluation studies conducted in 12 experimental groves during the past 3 years have demonstrated that only 14 of these biologic agents are sufficiently common or important to be worthy of consideration. These studies have shown that natural control is largely seasonal and dependent on the density of host insects, host mites, pathogenic agents, insect parasites and predators. It was also found that the degree of natural control is greatly affected by the insecticidal, fungicidal, and nutritional sprays applied to citrus.

METHODS OF SURVEY.—The occurrence and distribution of diseases, parasites and predators of citrus insects and mites were determined by extensive collecting and rearing. Although most of the rearing work was confined to material collected from 12 experimental groves, many of the data on distribution were provided by personnel of Dr. R. M. Pratt's survey project, who regularly observe insect and plant disease conditions in 130 widely distributed citrus groves.

Estimates of host insect populations included in figures 1, 5, 7 and 9 were based on the number of infested units of randomly selected leaves or fruit. For purple scale and Florida red scale a 50-leaf sample was randomly picked and observed in the laboratory under a dissecting microscope using the quarter-leaf method proposed by Griffiths & Thompson (1949). A sample composed of 32 randomly selected terminal leaves was examined in the grove with a 3× binocular loupe for infestations of the citrus red mite. A similar leaf sample examined in the grove with a 10× hand lens for leaf infestations of the citrus rust mite was supplemented by a sample of 20 randomly selected fruit examined in the same manner for fruit infestations of this mite.

Comparative populations shown in figures 3, 4, 6, 8 and 10 were obtained from samples of 40 leaves randomly selected at the rate of 10 leaves from each of 4 trees in a test block or grove. The samples were examined in the laboratory under a dissecting microscope and the various insects and mites were tabulated in the following manner: total healthy adult female scales, total healthy citrus red mites, total living predators, total diseased scales or mites and total parasite mummies or emergence holes.

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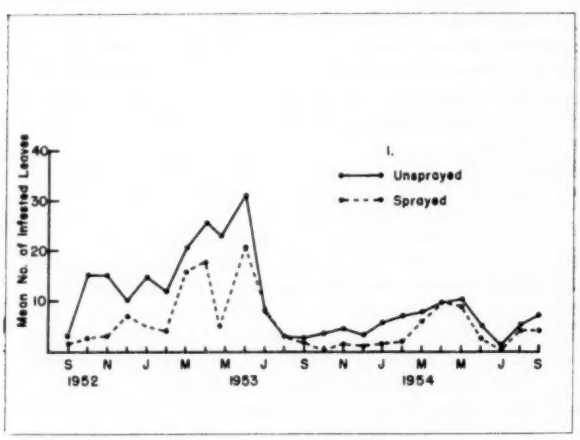

FIG. 1.—Mean number of leaves, in 50-leaf samples, infested with *Lepidosaphes beckii* (Newm.) in sprayed and unsprayed blocks of Valencia orange trees at Dundee, Florida from September 1952 to September 1954.

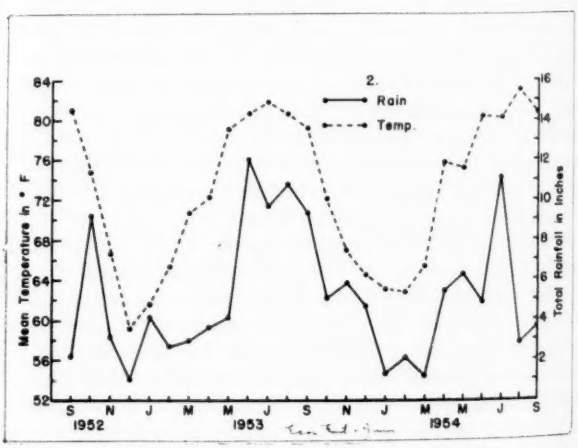

FIG. 2.—Mean monthly temperatures and total monthly rainfall at Mountain Lake, Florida (near Dundee) from September 1952 to September 1954.

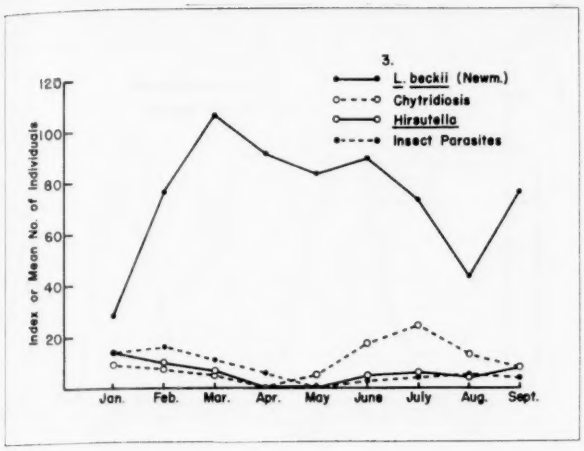

FIG. 3.—Mean number of adult female *Lepidosaphes beckii* (Newm.) and mean number of parasitized scales (index for *Hirsutella*) on 40-leaf samples in three unsprayed blocks of Valencia orange trees from January to September 1954.

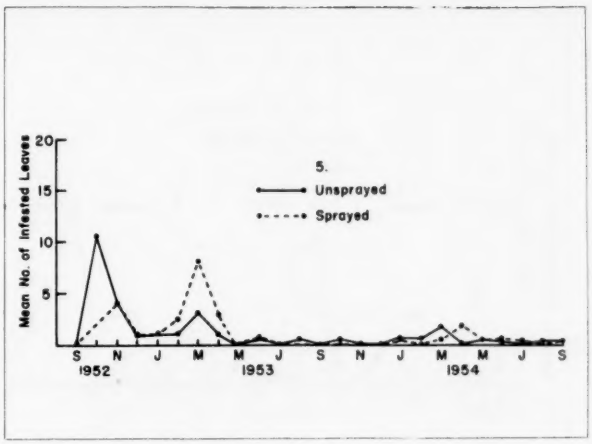

FIG. 5.—Mean number of leaves, in 50-leaf samples, infested with *Chrysomphalus aonidum* (L.) in sprayed and unsprayed blocks of Valencia orange trees at Dundee, Florida from September 1952 to September 1954.

Rust mites were counted when less than 100 to a leaf were seen but recorded as 100 when 100 or more were present. *Hirsutella besseyi* Fisher was indexed 1 for rare, 2 for common and 3 for abundant on each leaf and the index numbers were totaled for each sample.

Data on the relationships of six-spotted mites and predators cited in figures 11 and 12 were obtained from samples consisting of 20 of the most heavily damaged leaves observed in each test plot or grove. These leaves selected from 10 or more trees were observed in the laboratory under a dissecting microscope. All predators were counted and the six-spotted mites were indexed as to abundance. Leaves supporting 1 to 50 mites were indexed as 1, those supporting 51 to 100 mites as 2 and those supporting 101 or more mites as 3. The index numbers were totaled for the sample.

Temperature and rainfall data in figure 2 were taken from the United States Weather Bureau monthly reports for Mountain Lake, Florida. This station was located about 4 miles south of the Dundee test plot and was the nearest official weather station.

RESULTS OF SURVEY.—*Purple Scale.*—Purple scale, by far the most common scale insect injurious to citrus in Florida, is distributed throughout the citrus growing areas of the state and is found in nearly every citrus grove. Populations in untreated groves reach a maximum in late spring and early summer. Density of the scale is greatest on the limbs and on inside foliage and fruit, with the lightest populations occurring on outside foliage and fruit.

Two fungus diseases associated with the purple scale have been identified. They are a mycosis of crawlers caused by or associated with the fungus *Hirsutella besseyi* (1950) and chytridiosis, a disease caused by an endoparasitic chytrid, *Myiophagus* sp., that infects the nymphal instars and mature female scales. Chytridiosis was identi-

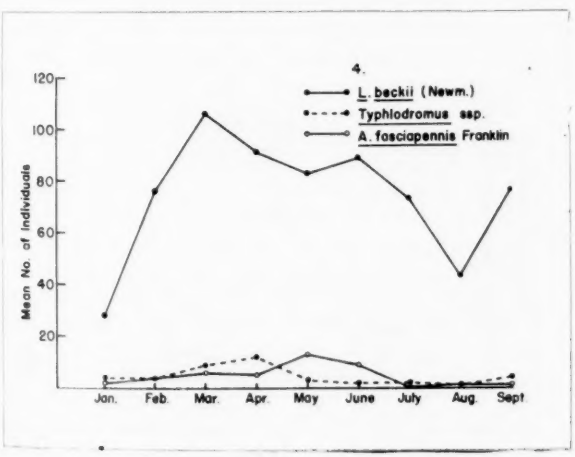

FIG. 4.—Mean number of adult female *Lepidosaphes beckii* (Newm.) and predators on 40-leaf samples in three unsprayed blocks of Valencia orange trees from January to September 1954.

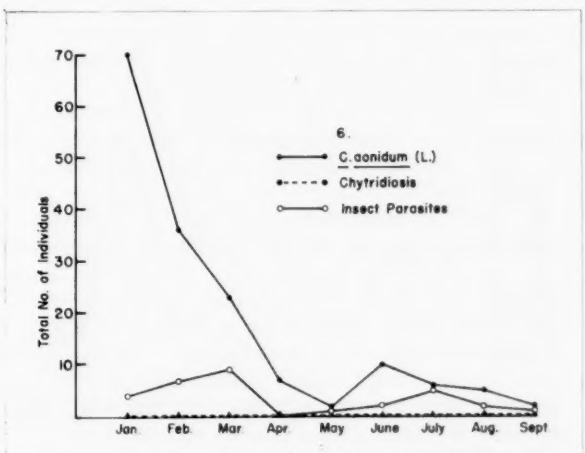

FIG. 6.—Total number of adult female *Chrysomphalus aonidum* (L.) and mean number of parasitized scales on 40-leaf samples in three unsprayed blocks of Valencia orange trees from January to September 1954.

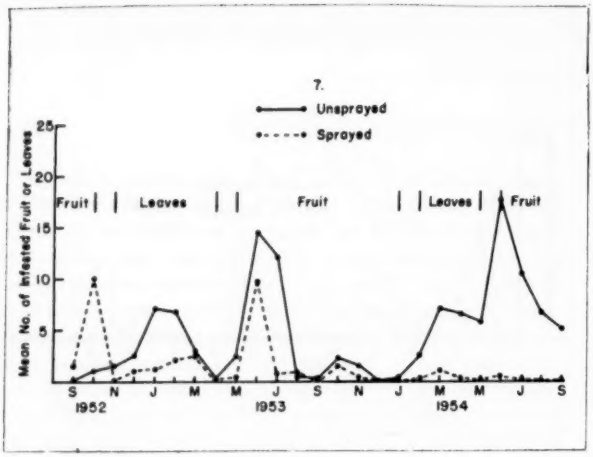

FIG. 7.—Mean number of fruit or leaves, in 20-fruit or 32-leaf samples, infested with *Phyllocoptruta oleivora* (Ashm.) in sprayed and unsprayed blocks of Valencia orange trees at Dundee, Florida from September 1952 to September 1954.

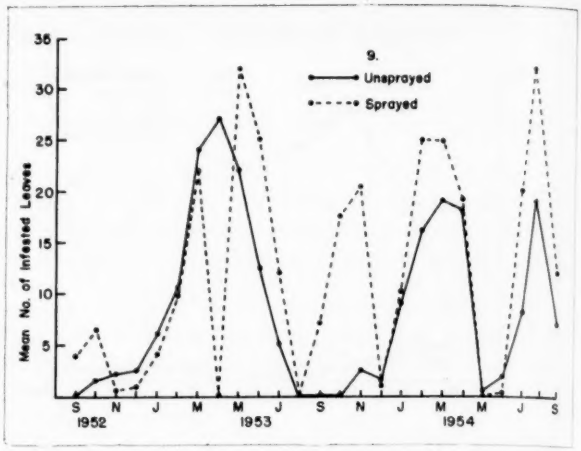

FIG. 9.—Mean number of leaves, in 32-leaf samples, infested with *Metatetranychus citri* (McG.) in sprayed and unsprayed blocks of Valencia orange trees at Dundee, Florida from September 1952 to September 1954.

fied by Fisher in 1948 and Fisher *et al.* (1949) outlined the life history of the chytrid on purple scale. These two entomogenous fungi have been discussed at length by Fisher (1950A), Fisher *et al.* (1949), Fisher & Griffiths (1951) and Griffiths & Fisher (1949, 1950). A third abnormal condition, caused by an unknown agent, has also been observed on purple scale. The scale body of affected late nymphs or mature females becomes light brown and flaccid, later changing to a dark brown and shriveling to a thin husk-like mummy.

Several species of parasitic hymenoptera have been reared from purple scale in Florida but only two eulophids, *Aspidiotiphagus citrinus* (Craw.) and *A. lounsburyi* (B. & P.), have been found in numbers. The latter species has been reared from second instar female and male nymphs, the former only from second instar females.

Many species of predatory insects and mites have been recorded feeding on purple scale, but only four are common and widespread. *Aleurodothrips fasciapennis* Franklin, a predatory thrips, is very common in the spring and early summer and is nearly always associated with scale infestations. Its eggs and pupae are frequently found under clumps of scale and in scale armors. Several genera and species of predatory phytoseiid mites are also associated with heavy populations of purple scale, particularly in the late winter and spring. One species, *Typhlodromus peregrinus* Muma is by far the most common. This species has fed readily on scale eggs and crawlers under laboratory conditions (Muma in press A). Other species have not been shown experimentally to feed on scale eggs and crawlers, but are consistently associated with scale. The twice-stabbed lady beetle, *Chilocorus stigma* (Say), has been shown by Muma (in press B) to feed rather extensively on purple scale even though it exhibits a low

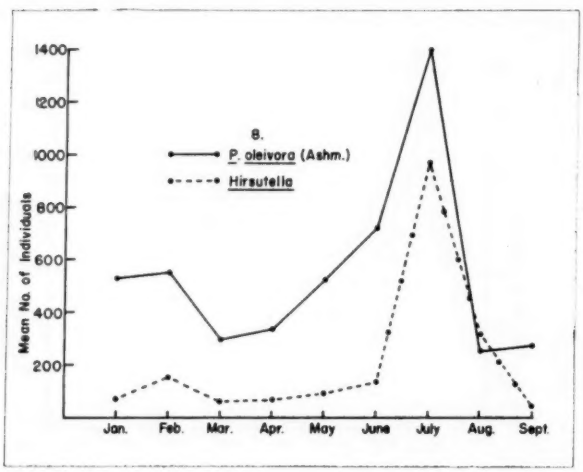

FIG. 8.—Mean number of *Phyllocoptruta oleivora* (Ashm.) and mean number of mites infected with *Hirsutella thompsonii* Fisher on 40-leaf samples in three unsprayed blocks of Valencia orange trees from January to September 1954.

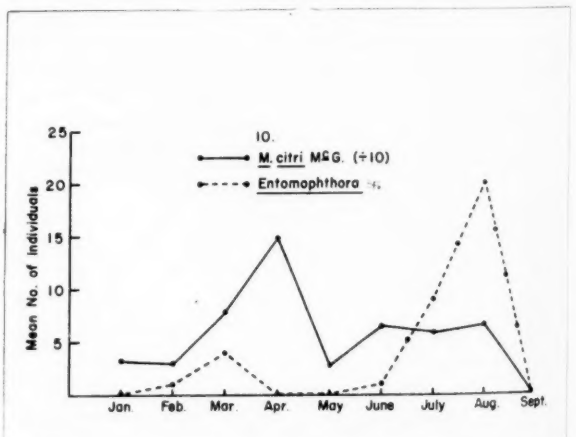

FIG. 10.—Mean number of *Metatetranychus citri* (McG.) and mean number of mites infected with *Entomophthora* sp. on 40-leaf samples in three unsprayed blocks of Valencia orange trees from January to September 1954.

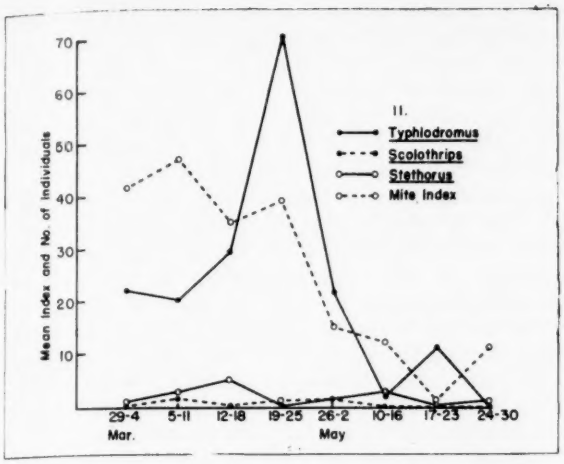

FIG. 11.—Mean *Eotetranychus sexmaculatus* (Riley) index and mean number of predators in five groves during the spring of 1953. Data obtained from samples of 20-infested leaves from each grove.

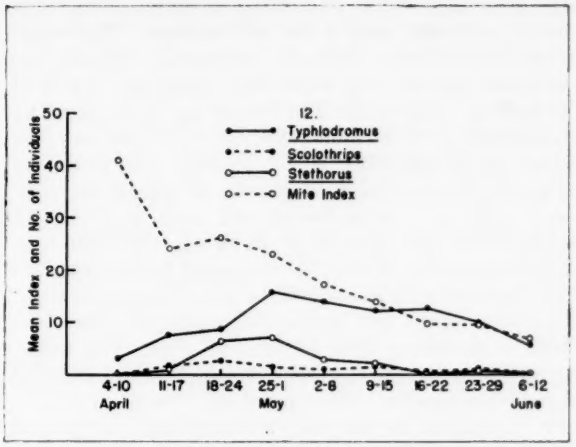

FIG. 12.—Mean *Eotetranychus sexmaculatus* (Riley) index and mean number of predators in nine groves during the spring of 1954. Data obtained from samples of 20-infested leaves from each grove.

fecundity when purple scale constitutes a large proportion of the diet. Occasionally large populations are found in groves where purple scale is the predominant injurious scale. Another lady beetle, *Microweisea coccidivora* (Ashm.) has also been commonly observed feeding on purple scale.

Representative data on the natural control of purple scale are given in figures 1, 2, 3 and 4. Figures 1 and 2 show that scale density is lowest following warm, wet weather and highest during dry or cool dry weather. This oscillation in density has been shown by Fisher *et al.* (1949) to be caused by scale mortality following epizootics of chytridiosis, which is most prevalent in warm wet seasons. Chytridiosis was prevalent even in the relatively dry summer of 1945 and scale mortality was high as shown in figure 3. Although *Hirsutella besseyi* is common during peaks of crawler activity and undoubtedly plays a part in the natural control of purple scale, it has not been proved to be directly associated with reductions in scale infestations. Insect parasites also appear to play a minor role in the natural control of this scale insect. During the fall of 1953 a ratio of parasitized to non-parasitized scale of 1 to 1.4 was found in one experimental block, but parasitization is normally much lower, about 1 to 60. Reductions in purple scale infestations that occur in late winter or spring usually are associated with populations of *Typhlodromus* spp. or *Aleurodothrips fasciapennis* as shown in figure 4. Although it has not been proved that these two predators are responsible for this reduction in scale density, the association has been demonstrated for the past 3 years and no other contributing factors have been discovered. Reductions in scale infestations, apparently due to the activities of these two predators, are striking but not enduring.

Natural control of purple scale in Florida is inadequate by comparison with insecticidal control, as shown in figure 1, despite the effectiveness of chytridiosis during the rainy season. Heavy infestations of scale occur in the late fall, winter and spring, and injury is evidenced in leaf drop, fruit drop and in some cases die-back, of infested twigs and limbs. Natural control of this scale is complicated by the use of insecticidal, fungicidal or nutritional sprays containing copper, zinc or sulfur which have a fungicidal effect on chytridiosis and *Hirsutella besseyi* as shown by Griffiths & Fisher (1949, 1950) and Fisher & Griffiths (1951).

Florida Red Scale.—Florida red scale, although not so common or widespread as purple scale, is a major injurious insect on citrus in the state. It is more common in the East Coast and Central citrus areas, but is found in about 60 per cent of all groves. Large populations usually occur in the fall and spring, with spring populations generally largest. Infestations are restricted to the fruit and leaves, the greatest density of scale often occurring on outside foliage and fruit. Frequently one or two limbs of a tree will be much more heavily infested than the others.

Two diseases of this scale have been identified. Chytridiosis has been recorded by Fisher *et al.* (1949) and a mycosis of crawlers, caused by the same fungus as that found on purple scale crawlers, has also been observed. A third abnormal condition caused by an unknown agent has been recognized. Affected scales become watery, develop a brown spot usually near a margin, turn a greasy brown in color and dry out to a mummy-like husk. This condition is referred to in this paper as brown-spot disease.

Eight species of parasitic hymenoptera have been reared from Florida red scale, but only four,— three eulophids and one encyrtid,—are common. The encyrtid, *Pseudhomalopoda prima* (Gir.) infests mature female scales whereas the most common eulophid, *Prospaltella aurantii* (How.), infests second instar female nymphs. Second instar male and female nymphs are also attacked by *Aspidiotiphagus lounsburyi* and *A. citrinus*, which are generally more common in male nymphs with the former much more prevalent than the latter.

Although a large number of predatory insects and

mites have been observed feeding on or associated with Florida red scale, only a few are common. The twice-stabbed lady beetle, *Chilocorus stigma* and *Microweisea coccidivora* are the only coccinellids commonly found in infestations of this scale. The former has been shown by Muma (in press B) to prefer this scale to purple scale, the latter's food preference is not known. The predatory thrips, *Aleurodothrips fasciapennis*, is almost as commonly associated with Florida red scale as it is with purple scale. One species of lacewing, the trash-bug, *Chrysopa lateralis* (Guer.), is a general feeder that has been demonstrated to complete a life cycle on this scale. Several laelaptid and phytoseiid mites are also consistently associated with Florida red scale. Two mites, *Typhlodromus peregrinus* and *Cosmolaelaps* sp., have fed on red scale crawlers in the laboratory.

A comparison of the climatological data in figure 2 with the population data in figure 5 shows that in unsprayed groves there is no distinct relationship between Florida red scale populations and rainfall. This indicates that entomogenous fungi probably do not play a major role in the natural control of this scale. Additional circumstantial evidence supporting this supposition is presented in figure 6 wherein the only natural control factors oscillating with scale density are the insect parasites. Natural control of this scale by insect parasites has been previously reported by Griffiths & Thompson (1947) and Griffiths & Stearns (1947) who found that the use of DDT on citrus in Florida increased infestations and reduced parasitization. It is probable that chytridiosis, the little known brown spot disease, and one or more of the known predators also contribute to natural control of Florida red scale but insufficient data are available to show such a contribution.

Natural control of Florida red scale on citrus in Florida is usually adequate and comparable to insecticidal control as shown in figure 5. Infestations in unsprayed plots have been as low or lower than those in sprayed plots for the past two years.

Citrus Rust Mite.—The most common injurious mite on citrus in Florida is the rust mite. Although distributed on citrus through out the state, it is more injurious in the south-central and west coast areas than elsewhere. It is second only to purple scale in common occurrence and is regularly found in about 66 per cent of the groves. The heaviest populations occur in winter, spring and summer, most frequently on young foliage and fruit on the outside on the trees but occasionally on old inside foliage and inside fruit.

A fungus disease of rust mites was first reported by Speare & Yothers (1924) and Yothers & Mason (1930). Later, a fungus described by Fisher (1950) as *Hirsutella thompsonii* Fisher was reported to cause, or be associated with, an epizootic of rust mites (Fisher *et al.*, 1949A). Although it has never been proved that the earlier reported disease was associated with the same fungus as that described by Fisher, the described symptoms were similar.

Yothers & Mason (1930) observed a number of predatory insects feeding on rust mites but none of the insects was sufficiently common to be of value in reducing rust mite populations. Recently the mealywing, *Coniopteryx vicina* Hagen, has been found to occur in numbers sufficient to be of value in natural control. The species is present in large to moderate numbers in most rust-mite-infested groves but does not occur on the East Coast. Under laboratory conditions, adults of the species have fed voraciously on rust mites. The larvae have not been demonstrated to feed on this species although they are known to feed on scale crawlers and six-spotted mites.

Figures 2 and 7 show that the large rust mite populations that develop in late spring and early summer are greatly reduced following rains. The relationship of these reductions in rust mite infestations to epizootics of *Hirsutella thompsonii* is shown in figure 8. No known natural control factors have been found to be associated with reduced infestations of rust mites that usually occur in the early spring. Although *Coniopteryx vicina* is common in groves heavily infested with rust mites, no oscillations in mite density have been attributable to this insect.

Natural control of rust mites by mycosis extends into the fall and early winter. With the advent of dry weather in the late winter and spring, heavy infestations develop, causing extensive damage to fruit. As indicated in figure 7, natural control of this mite is not comparable to that obtained with sulfur. Unfortunately, however, sulfur, as shown by Griffiths & Fisher (1949, 1950), has a fungicidal effect on the entomogenous fungus, which further reduces the effectiveness of natural control.

Citrus Red Mite.—The citrus red mite is the most common of the three species of spider mites that infest citrus in Florida. It is generally distributed throughout the citrus growing areas, where it is found in about 60 per cent of the groves. Heavy infestations usually occur in late winter and spring, occasionally in the summer and fall. The mites feed on the leaves, fruit and twigs, but are usually most numerous on the upper surfaces of the terminal leaves.

Two fungi are known to infect this mite. The most common of these is a species of *Entomophthora* that has been identified and discussed by Fisher (1951); the other is a species of *Hirsutella* recently found in a single grove on the East Coast. A third condition commonly seen but not as yet identified with a causal agent appears to be a dysentery. Affected mites are flaccid and usually stuck to the leaf surface in a smear of black resinous material that appears to have exuded from the anus.

The predator most commonly associated with heavy infestations of the citrus red mite is the trash bug, *Chrysopa lateralis*. It is a general feeder and has been demonstrated by Griffiths & Thompson (1950) to feed readily on this mite. These workers also observed two coccinellids, *Olla abdominalis* var. *plagiata* (Say) and *Stethorus utilis* Horn, and a naked species of *Chrysopa* feeding on this mite, but these predators have not been common in recent citrus red mite infestations. In addition to the above previously recorded species, several predatory mites of the genus *Typhlodromus* are also commonly found associated with this mite.

Although numbers of trash bugs frequently accompany large infestations of citrus red mites, it has not been possible to attribute any reduction in mite density to these insects. Under laboratory conditions, *Typhlodromus floridanus* Muma has decimated purple mite infestations, but this has not been observed in the groves. *Entomophthora* sp. appears to be the limiting factor in natural

control of the citrus red mite, but its efficiency in reducing mite populations is dependent on weather conditions as indicated in figures 2 and 9. Citrus red mite infestations are lowest during and immediately following periods of high rainfall and after warm winters as reported by Pratt & Thompson (1953). Figure 10 shows that epizootics caused by *Entomophthora* sp. occur during or immediately following periods of increased rainfall and appear to be responsible for reductions in mite density. The cause for the inverse relationship of winter temperatures to spring citrus red mite infestations is not known.

Natural control of the citrus red mite is greatest during the summer and fall months, due to the efficiency of *Entomophthora* sp. In the winter and spring, however, injurious populations are frequently encountered and chemical controls must be applied to prevent leaf drop. Natural control of this mite is complicated by the fact that sulfur and copper, two chemicals commonly used on citrus, apparently reduce the efficiency of the fungus as suggested by Thompson (1940, 1944).

Six-spotted Mite.—Although heavy infestations of the six-spotted mite are not annual occurrences, the species is very injurious when present in large numbers. Fortunately, as reported by Pratt & Thompson (1953), this mite has been a major problem in Florida in only 6 of the last 19 years. These two workers also showed that infestations reached a peak in May or June and rapidly decreased to non-injurious levels in early summer, that infestations were more severe on the West Coast, and that infestations were more common and severe on grapefruit than on orange trees.

A mycosis of six-spotted mites has been reported by Fisher (1954) but the fungus has not been identified.

Ten species of predators, six insects and four mites, have been recorded feeding on this mite. Most of them are not widely distributed or consistently present in infestations, and must be considered to be of minor importance. Three species, *Typhlodromus floridanus*, *Stethorus utilis*, and a thrips (*Scolothrips sexmaculatus* (Perg.)) are nearly always associated with infestations of six-spotted mites and merit consideration.

The effects of weather on six-spotted mite infestations have been reported and discussed by Pratt & Thompson (1953), who found that severe infestations were correlated with low temperatures during the preceding December. These workers also reported that abundant rains produced mortalities of six-spotted mites during the spring of 1953.

Although six-spotted mites did not attain high populations during 1953 and 1954, sufficient numbers were present to permit an evaluation of the natural control factors. Figures 11 and 12 present data accumulated on predator and six-spotted mite populations. It would appear from these data that predators play an important part in reducing six-spotted mite infestations, and that *Typhlodromus floridanus* is the most important predator. It must be stated, however, that these figures, although obtained in moderately to severely damaged groves, probably are not representative of conditions that would exist during years of severe six-spotted mite infestations. No diseased mites were encountered in any of the samples.

Natural control of six-spotted mites by large populations of predators is effective in seasons preceded by a warm December. Insecticidal control is necessary, however, in seasons preceded by a cold December.

SUMMARY.—Results of a survey of the pathogenic agents, parasites and predators of the more important injurious insects and mites on citrus in Florida is reported. A preliminary evaluation of these factors has revealed some of the apparent causes for the relatively high degree of natural control known to exist in some Florida groves.

Two parasitic fungi, predatory mites and a predatory thrips appear to be the important factors in natural control of purple scale. The highest degree of natural control is obtained during the summer rainy season when the fungi are effective, but control is not comparable to that obtained with insecticides.

Natural control of Florida red scale apparently is maintained by insect parasites, with parasitic fungi and predators being of secondary importance. The degree of control in unsprayed groves is usually adequate and comparable to insecticidal control in sprayed groves.

Parasitic fungi seem to be important factors in natural control of the citrus rust mite and citrus red mite. Predators, although common, appear to play a minor role. Citrus rust mite infestations are greatly reduced in late summer and early fall following epizootics of a parasitic fungus but apparently are not affected by large populations of a coniopterygid. The same is true of citrus red mite infestations, which are controlled by a parasitic fungus in summer and early fall but do not respond to concurrent infestations of the trash bug. Natural control of these two important mites is not comparable to that obtained with acaricides except during periods of high fungus density.

Three predators—a mite, a lady bettle and a thrips—appear to be the principal natural control factors influencing six-spotted mite infestations. These predators have been common during the light infestations of the past 2 years, but no data have been collected when six-spotted mite infestations were severe. Natural control of this mite is usually adequate following warm winters but inadequate following cold winters.

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Measuring Wireworm Reactions to Soil Insecticides by Tagging with Radioactive Cobalt[1]

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Wireworm control is necessary for the successful production of some crops in almost every important agricultural region of the world. The techniques used to follow wireworms in the soil, however, have always left much to be desired. Until recently the methods used were very artificial and the wireworm habitat had to be interrupted so that the insect could be studied. This problem has been solved by recent developments in the use of radioactive isotopes. By using radioactive nuclides whole animals such as insects can be traced. The insect is allowed to move about in any direction, and the natural environment is no longer altered as in other procedures.

The primary purpose of the work reported here was to study wireworm reactions to certain soil insecticides by the use of this new technique. Work similar to this was carried out by Arnason *et al.* (1950), Lebedev (1950), and Fuller *et. al.* (1950). Both Arnason and Fuller used cobalt 60 to tag wireworms, and made both internal and external applications of the radioactive sources. Their work was conducted in Saskatchewan, Canada. Lebedev, a Russian, also tagged soil-inhabiting insects for studying their habits, but further information on his work is not available. These three papers represent the use of isotopes on soil-inhabiting insects. However, other isotopes and other insects have been used in recent work summarized by Dahm (1953).

MATERIALS AND APPARATUS.—Wireworms of the genus *Melanotus* were used. These were collected by Dr. Walter Kulash near Raleigh, North Carolina, shipped to Iowa State College by Air Express, and individually placed in salve tins. These tins were stored in the refrigerator at about 40° F. until used.

Experiments were carried out in the greenhouse in wood boxes 10 inches wide, 20 inches long, and 8 inches tall, filled with 50 pounds of loam soil. The soil was mixed in a drum turned by an electric motor.

The insecticides used were 25% lindane (Geigy), 25% aldrin (Shell), 50% dieldrin (Shell), and 25% heptachlor (Velsicol). All were wettable powders and pyrophyllite was added to each of them as a diluent so as to approximately double their volumes before use.

Radioactive cobalt 60 wire was used to tag the wireworms. The wires were 1 millimeter long and 0.5 millimeter in diameter. They were gold plated to prevent corrosion of the cobalt, and had an initial activity of about 0.07 millicurie.

The larvae were placed in a simple knockout-cage consisting of a 4-inch length of $\frac{1}{4}$-inch glass tubing.

A Nuclear Instrument and Chemical Company 1615B Count Rate Meter was used to follow the tagged wireworms. The radiation detector was a Tracerlab TGC-8 high-efficiency gamma Geiger-Müller tube.

The sources used were calibrated for different soil levels in a 1-liter graduate calibrated in inches.

A film badge was worn to detect any absorption of radiation by the body of the investigator.

PROCEDURE.—Each source was calibrated for various depths of soil. The source was placed at the bottom of the graduate and soil placed on top of it. A reading was taken for every inch of soil added, and a graph was made from these readings. Thus soil depth was plotted against the counts per minute readings. The radiation detector was

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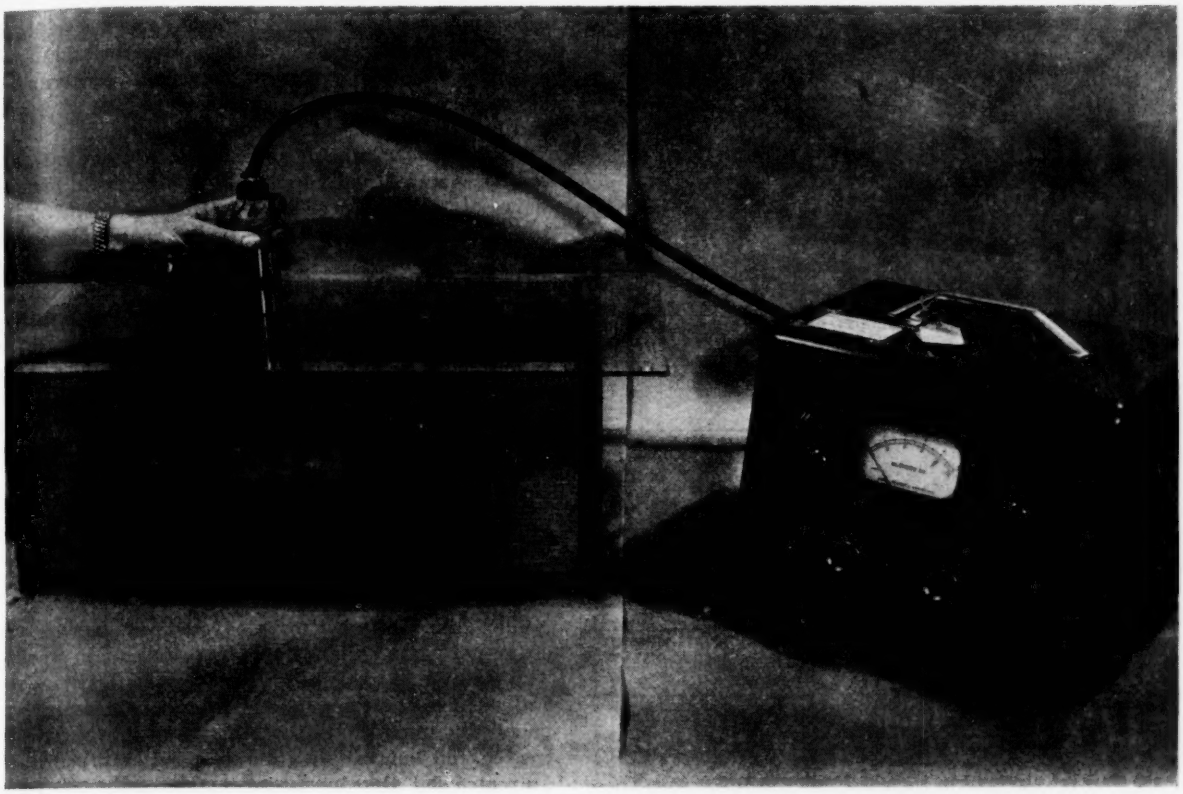

FIG. 1.—Count rate meter and radiation detector held in position ready to take a reading. The glass plate over the soil box shows the grid cross-marked in inches.

held at a constant distance from the soil surface as the soil was added to the graduate cylinder. A graph was made for each source in this manner. When radiations of a certain intensity were recorded in the experiments, this graph showed the exact depth of the larva.

The soil and insecticide were mixed in the drum for 320 revolutions at 32 revolutions per minute. The four insecticides were used at the rate of 5 pounds of actual toxicant per acre on a weight basis. The soil box was divided into two parts before filling. One end was filled with untreated soil, and the opposite end with insecticide-treated soil. Pyrophyllite was mixed with both halves of the soil. The temporary dividing partition was removed after filling so that no physical barrier separated the two lots of soil.

In tagging, a wireworm was placed in a "knockout" cage and carbon dioxide added until the insect was inactivated. The most satisfactory knockout cage was simply a 4-inch length of $\frac{1}{4}$-inch glass tubing closed with fine screen on one end, and connected to a CO_2 cylinder by a rubber tube fitted over the other. The larva was then placed in a large glass tray where all tagging took place. A notched piece of cardboard was held perpendicular over the posterior end of the worm so that only the caudal segment of the abdomen was left showing. This protected the remainder of the insect's body from the vinylite spray used to attach the cobalt. The caudal segment was then cleaned with a camel's-hair brush and sprayed with vinylite cement. After 4 to 5 seconds the cobalt wire was placed on the dorsal surface of the caudal segment with a pair of forceps and given another spray of cement. The tagged larva was then placed in its individual salve box until the start of the experiment.

One tagged larva was placed in each of the four soil boxes containing soil insecticide mixtures and in the untreated control box. The time and place of entry into the soil were recorded. The larvae were allowed to enter the soil by free choice. A glass grid cross-marked in inches was placed over the box and the radiation detector was moved over the glass until the point of highest radiation intensity was found (Figure 1). This located the larva in the horizontal plane, and a record of its position was taken from the grid. The counts-per-minute reading on the count rate meter was also recorded, and the depth figured from the prepared calibration graph. This procedure was used for each box.

Readings were taken about every hour for the first 2 days and less frequently after that. Therefore any changes in position by the larva represented the minimum distance traveled during the period between readings. The experiment was terminated at the end of 4 days.

After the experiment was ended the wireworms were removed from the soil, their tags removed and cleaned in acetone, and the larvae placed in individual salve tins for further observation.

RESULTS.—Wireworm reactions to four soil insecticides

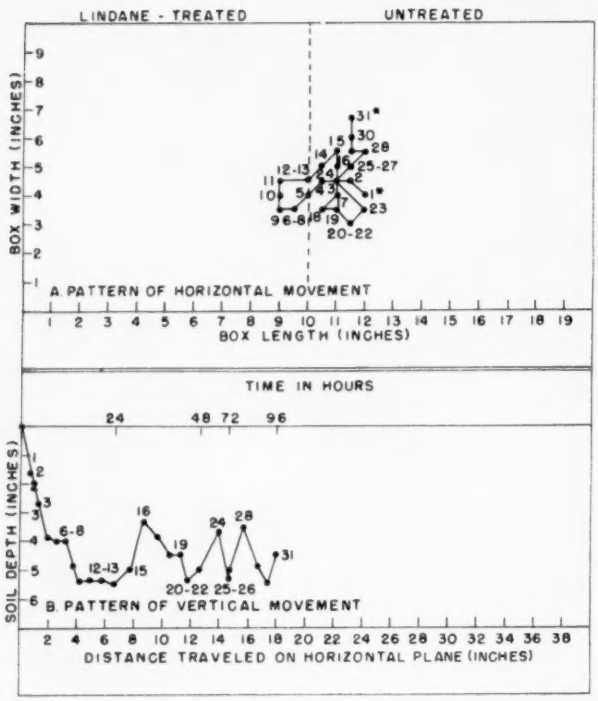

FIG. 2

are shown by graphs of their movements in the soil. These graphs show typical patterns of both the horizontal and the vertical movements made by individual wireworms.

The soil temperature was maintained between 75° and 85° F. at all times, and the soil moisture was maintained between 25 and 35 per cent, calculated on a wet soil basis.

Vertical movements of all the experimental larvae generally followed a diurnal movement pattern. The larvae were deepest in the soil during the hot afternoons and nearest the soil surface in early morning.

Larval movements in the lindane-treated: untreated soil boxes were usually limited to a small area. Figure 2 shows a total distance of 18 inches traveled in 4 days. This wireworm ventured into the treated area, but soon returned to the untreated soil and remained there until the end of the experiment.

Wireworm movements in the aldrin-treated: untreated soil boxes occupied an area less than one-fourth the total. Figure 3 shows the larval entrance into the treated soil, its migration to the untreated soil, a second entrance into the treated soil, and a second escape into the untreated area of the box. The total distance traveled was 31 inches.

Horizontal movements in the dieldrin-treated: untreated soil boxes usually covered a larger area than was the case with the preceding two treatments. Figure 4 shows a total distance traveled of 41 inches, and only a short period of time spent in the treated soil.

The horizontal movements covered over half the area of the box containing heptachlor-treated soil in all cases. The directional changes of these wireworms were generally sharp, and the angles of over 90 degrees were frequent. Figure 5 shows that this larva moved into the treated soil but within a few hours it returned to the untreated soil. One day later it reentered the treated soil but returned to the untreated area just before the experiment was ended.

Horizontal movements in the untreated control boxes

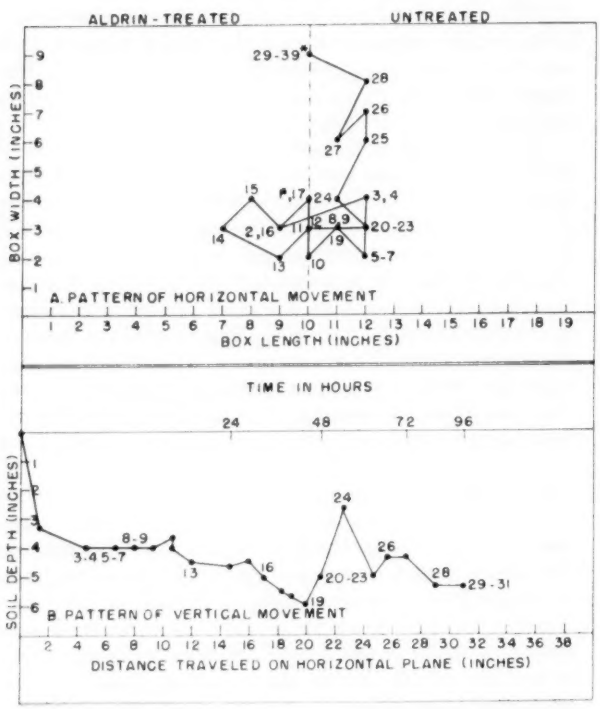

FIG. 3

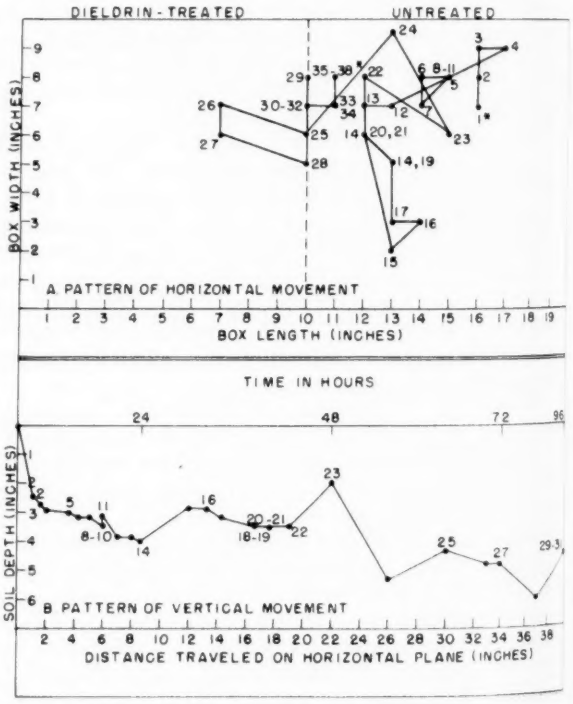

FIG. 4

covered from over half to almost the entire area. Individual movements were quite long and random in nature, as shown in figure 6. No one area of the control box seemed to be the most favorable spot, and the larvae moved freely in both halves of these boxes. Total distance traveled by the larva shown in figure 6 was 50 inches.

DISCUSSION.—A summary of the data of the 6 replications for the 4 treatments and the untreated control is presented in table 1.

Table 1 shows that the average total distance traveled by wireworms in lindane-treated soil and aldrin-treated soil were the same, i.e., 33 inches. These two treatments resulted in the most pronounced slowing of larval movements. In order to save space and reduce cost the movements of only 5 of the 30 test insects are presented graphically.

Table 1.—Distances traveled by wireworm larvae (*Melanotus* sp.) tagged with cobalt 60 and placed in boxes of loam soil containing four different insecticides. Ames, Iowa, 1954.

TREATMENT	REPLICATION	TOTAL DISTANCE TRAVELED IN INCHES AFTER: 2 Days	4 Days
Lindane	1	23	28
	2	15	26
	3	35	41
	4	24	36
	5	33	47
	6 (Fig. 2)	12	18
	Average	24	33
Aldrin	1	13	20
	2	23	32
	3 (Fig. 3)	21	31
	4	21	30
	5	32	45
	6	28	41
	Average	23	33
Dieldrin	1 (Fig. 4)	22	41
	2	17	25
	3	31	50
	4	19	26
	5	33	51
	6	43	70
	Average	27	44
Heptachlor	1	29	51
	2	17	24
	3	23	32
	4	29	45
	5 (Fig. 5)	23	41
	6	33	42
	Average	26	39
Untreated	1	20	32
	2	27	37
	3 (Fig. 6)	29	50
	4	35	57
	5	38	65
	6	46	70
	Average	32	52

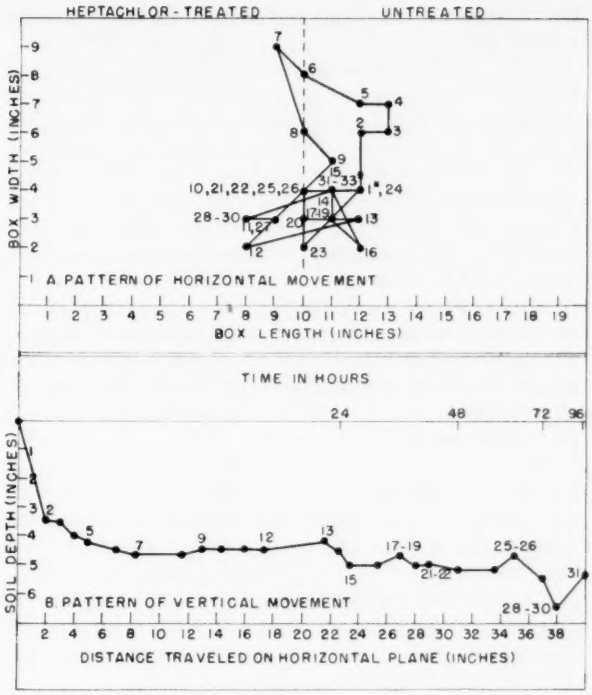

FIG. 5

Dieldrin-treated soil and heptachlor-treated soil resulted in more wireworm activity. Some of the more active larvae appeared to show escape reactions indicated by bizarre patterns of movement and sharp directional turns, particularly in heptachlor-treated soil. Most of

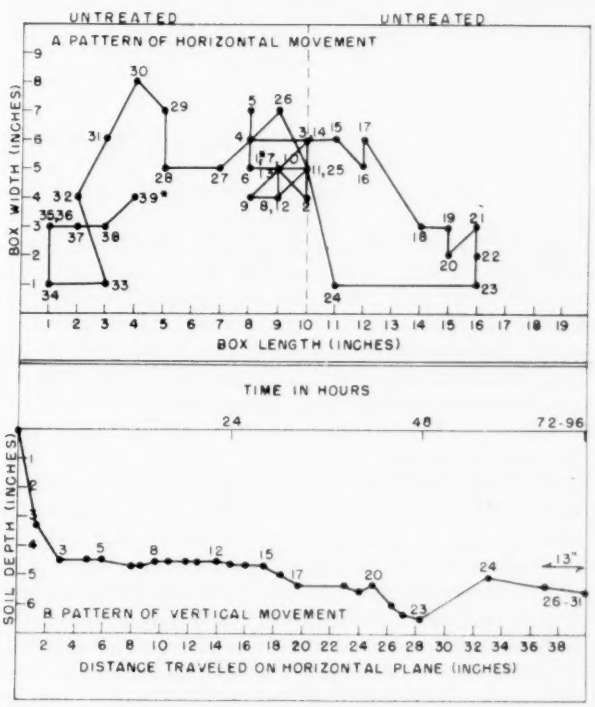

FIG. 6

the movements were long and random in the untreated control soil.

Fifteen of the 24 experimental larvae moved into the treated soil and back out again within a few hours. These reactions seem to indicate a repellent action by these four toxicants. In a few cases the larvae stayed in the treated soil and died or were moribund when recovered. A few also entered the untreated soil and failed to enter the treated soil during the course of the experiment. In general the experimental wireworms tended to avoid the insecticide-treated areas.

SUMMARY AND CONCLUSIONS.—A study was made of wireworm reactions to four soil insecticides by tagging the larvae with radioactive cobalt. A radioactive wire was cemented to the dorsal surface of the caudal segment of a wireworm, after which the larva was placed in soil for "observation."

The wireworm was located directly below the radiation detector when the point of highest radiation intensity was found. This intensity was recorded in counts-per-minute, and a graph of soil depth versus intensity was then used to determine the vertical position of the wireworm from the count rate. The horizontal position was recorded from a glass grid placed over the soil box. In this manner both vertical and horizontal movements were recorded simultaneously.

The following conclusions were reached as a result of this study:

Soils treated with aldrin or lindane resulted in the greatest reductions in wireworm movements of the four insecticides tested.

Dieldrin-treated soil showed the least reduction in wireworm movements of the four insecticides.

Heptachlor-treated soil showed reductions in wireworm activity intermediate between those of lindane and aldrin on one extreme, and dieldrin on the other.

Untreated control soil appeared to be the optimum medium for wireworm movements.

There is ample evidence that all four of these insecticides were quite toxic to wireworms under the conditions of these experiments. First, all the test insects that entered treated soil and stayed there were dead or moribund when the experiment was terminated after 4 days. Second, 2 weeks after the experiment ended all of the wireworms from boxes containing treated soil were dead, even though they had failed to enter the treated area. This suggests a fumigating action. The control wireworms in the boxes containing untreated soil throughout were alive and apparently normal 2 weeks after the experiment was ended.

Radioactive tagging is a practical and useful means of following wireworms in the soil. The development of this technique opens the way for extensive research on soil-dwelling insects without disturbing their natural habitat.

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Control of the Corn Earworm in Florida[1]

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Most of the sweet corn grown in Florida is marketed as fresh corn. Satisfactory returns to the grower of fresh corn depend upon his ability to produce a worm free, unclipped, fancy grade of corn. Thus, the standards a control measure must meet in Florida are much more rigid and difficult to attain than in areas where sweet corn is grown to be marketed for processing.

Almost all of the commercial plantings of sweet corn are made in the peninsular part of the State. For the purposes of this discussion, this section is divided into three fairly well defined areas (Fig. 1). They are designated as the southern, central and northern areas. About 75 per cent of the Florida acreage of sweet corn is grown in the southern area. Approximately 85 per cent of the acreage in this southern area is planted on the peat and muck soils along the southeastern shore of Lake Okeechobee, and the remaining 15 per cent on the mineral soils of the east and west coasts. The central area grows about 18 per cent of the State's acreage, and about 4 per cent is grown in the northern area. The remaining 3 per cent is reported by the Bureau of Agricultural Economics under the inclusive term "Miscellaneous." The crop season for sweet corn in the southern area differs very widely from that of the central and northern areas, while these last two areas vary somewhat from each other. For this reason, corn earworm populations, control practices, and results are discussed for the areas as outlined rather than for the State as a whole.

In the southern Florida area sweet corn is planted in the early fall, through the winter and into the spring months. Harvesting usually begins about October 15 and continues to the first of June. In this area the corn-silk fly, *Euxesta stigmatias* Loew, is usually abundant enough to require control during the later fall and early spring months. During the fall and winter months populations of the corn earworm, *Heliothis armigera* (Hbn.), are usually low, but they gradually increase during the spring

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months to such a point that satisfactory control is very difficult to obtain. The seasonal abundance of the corn earworm and the close relationship between earworm populations and the degree of control is demonstrated by data collected during the 1952–53 growing season by W. H. Thames of the Everglades Experiment Station (Table 1).

Three types of equipment are used in the southern Florida area to apply insecticides for earworm and silk fly control. The estimated percentage of the acreage of the area treated by each of the types of equipment are: 5 per cent by airplane dusters, 44 per cent by ground sprayers, and 51 per cent by ground dusters. The growers using airplane and ground dusting equipment usually make 6 applications of 10 per cent DDT dust at 48-hour intervals at the rate of 30 to 50 pounds of dust per acre per application. During periods when the silk fly is abundant, growers use a dust containing 1.5 per cent parathion and 7.5 per cent DDT, or they may use a dust containing 2.5 per cent chlordane and 5 per cent DDT. During the latter part of the season when earworm populations are high, some growers make 12 to 14 applications of one of the 3 above mentioned dusts at 24-hour intervals. The growers who use ground spray equipment apply 3 to 4 quarts of 25 per cent emulsifiable DDT per acre. Since Northern and Southern blights (*Helminthosporium tercicum* and *H. maydis*) are serious diseases of sweet corn in the southern Florida area, the emulsifiable DDT is combined with nabam-zinc sulfate or nabam-manganese sulfate used for the control of these diseases. During periods of high earworm populations when the addition of mineral oil is required for satisfactory earworm control, the insecticide and fungicide are applied separately because the mixture of fungicide-oil-emulsifiable DDT is not compatible. All three method of applying insecticides give excellent control when earworm populations are low. After about May 15 earworm populations are usually high and the control is frequently unsatisfactory with all three of the methods of applying the insecticide.

Most of the sweet corn acreage in the central Florida area is planted to mature late in April, May and June. The probability of the occurrence of killing frost during December and January is too high to permit fall and winter planting in all but the counties on the Gulf Coast. The corn-silk fly and helminthosporium blights have not yet become economic factors in sweet corn production in this area. In the central area, as in the southern areas, a much larger proportion of the sweet corn is planted on peat soils than on the mineral soils. A survey of earworm

Table 1.—Effect of seasonal abundance of the corn earworm upon the degree of control obtained in the southern area of Florida.

TREATMENT[a]	PER CENT WORM-FREE EARS HARVESTED				
	Nov. 28	April 24	May 12	May 28	June 18
DDT Em. with mineral oil	100	99.8	—	88	95.8
DDT Em. without oil	100	99.8	—	74	75.0
DDT, 5%, dust	99	87.0	78	—	86.2
Parathion, 2%, dust	98	91.0	80	—	79.2
Untreated	84	35.0	18	0	0

[a] Six applications at 48-hour intervals for all treatments except the parathion and DDT dusts in the last trial which were applied 10 times at 24-hour intervals.

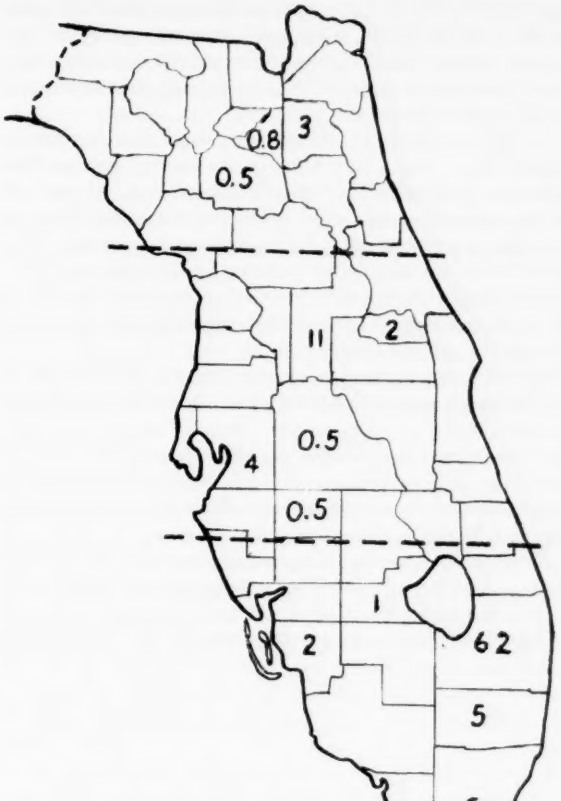

FIG. 1.—Distribution of acreage of sweet corn in Florida. Figures are per cent of total State acreage grown in the County in which the figures appear.

populations in the mineral-soil section of the central area, conducted during the 1954 growing season, indicated that the satisfactory control obtained prior to about May 15 is closely correlated with a low earworm population. Unsatisfactory control obtained after May 15 was likewise accompanied by a high earworm population. A similar survey in the peat-soil section indicated low earworm populations through the entire harvesting period, although corn was harvested during all of June. Sufficient information is not available at this time to form the basis for a statement on the seasonal earworm populations that may be expected in this peat-soil section of the central area.

Growers in the mineral-soils section use only ground dusting equipment, applying 10 per cent DDT dust at rates of 30 to 50 pounds per acre at 48-hour intervals. In the peat-soil section approximately 35 per cent of the acreage is treated by ground dusting equipment and 65 per cent by airplane dusting equipment. Most of the growers in this peat-soil section apply 10 per cent DDT dust at rates of 40 to 50 pounds per acre at 48-hour intervals. One grower has applied a 20 per cent DDT dust at 24-hour intervals. Another used a dust containing 2 per cent parathion, 3 per cent chlordane and 10 per

cent DDT. Others have applied 10 per cent DDT dust at 24-hour intervals. The low earworm egg count observed during the course of the survey indicates that large amounts of insecticide have been applied needlessly in this peat-soil section.

In the northern Florida area, sweet corn harvesting begins 2 to 3 weeks later than in the central area and the earworm population is reported to be high. About half of the acreage of this area is treated by ground dusting equipment and the other half by ground spraying equipment. Growers frequently fail to continue the insecticide applications through all of the silking period. As should be expected under such conditions earworm control is frequently unsatisfactory.

Current recommendations to growers are related to the earworm population, and are, in most cases, followed rather closely by the growers. Results obtained by the growers have been discussed. The variation in growers' practices during periods of high earworm populations emphasized the need for a more effective control measure for such periods.

During the 1954 growing season, work at the Central Florida and Everglades Stations was closely coordinated. Both Stations participated in the National Earworm Trials. The two Stations continued a study begun during 1953 of the effects on earworm control of the particle size of the DDT in dust formulations, the results of which are in press. An investigation of repellents for earworm control was initiated at both Stations. Although these tests of the six repellents used were screening-type tests, the preliminary results were promising enough to encourage further investigations. At the Everglades Station combinations of parathion and malathion with DDT in dust formulations and combinations of endrin, DDT and mineral oil sprays were investigated. At the Central Florida Station, a survey of the seasonal density of earworm eggs and larvae in commercial fields was initiated. Future research will be concerned with investigation of the effectiveness of repellents, combination of various insecticides with DDT in dust and spray formulations, and a continuation of the seasonal survey of earworm density in commercial fields.

ACKNOWLEDGMENTS.—Grateful acknowledgment is made to W. H. Thames for use of the data contained in table 1, and the information he supplied on growers' practices in the southern Florida area. Representatives of several companies formulating and marketing insecticides furnished useful information. A. N. Tissot and N. C. Hayslip have read the manuscript and made many helpful suggestions.

Factors Affecting Biological Control of Scale Insects on Texas Citrus[1]

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Biological control of citrus insects has been an important economic consideration in the Lower Rio Grande Valley citrus area during the past 20 years. Many growers who applied chemicals to control scale insects on an extensive basis found that their neighbors who used no chemical sprays profited as well and did not upset a predator-pest relationship in nature. Yearly spray operations seemed necessary once the grower started spraying. Other pests, for which the man did not spray, often developed to damaging numbers. Growers, thus, directed chemical control efforts against the two most important pests: the citrus rust mite, *Phyllocoptruta oleivora* (Ashm.) and Texas citrus spider mite, *Eutetranychus banksi* McG. At the present time, sprays are not commonly used in the area and dusts are the common means of applying chemicals for the control of these pests.

Apparently the first recognition in this area of the importance of citrus biological control was reported by Clark & Friend (1932). They reported that monthly applications of lime-sulphur solution to citrus trees produced a large increase of California red scale over that of unsprayed trees. Such increases were attributed to the detrimental effect of lime-sulphur against parasites and predators. The biological control era, however, really began following the hurricane of September, 1933, which destroyed the fruit crop and severely damaged many trees. Previously chemical control treatments had been directed against the citrus rust mite and the California red scale, *Aonidiella aurantii* (Mask.). The loss of the crop that year and the existing economic condition caused most growers to stop spraying. Weather and other conditions assisted beneficial insects to exercise their importance in the control of certain scale insects. Mr. S. W. Clark, entomologist at the substation during the transition period, studied the important parasites and predators of citrus scale insects from 1935 to 1937 and included the unpublished information in his 1937 annual report. Clark stated that *Aphytis chrysomphali* (Mercet), *Prospaltella aurantii* (How.), various *Prospaltella* species, *Aspidiotiphagus citrinus* (Craw), and various *Aspidiotiphagus* species were the most abundant citrus scale parasites present in the area. Clark had difficulty in obtaining identification at the time but realized the importance of the biological control means.

Practically no important information was taken from 1937 to 1949, except for the work of Roberts (1946). He reported that predators and parasites would keep aphids, scale insects, white flies, and thrips pretty well under check, if ants and mites were kept under control. DeBach (1951) visited the area in December 1949, and his report

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greatly assisted in the beginning of the present study.

Climatic conditions seemed to play a very important part in scale insect predator and parasite populations during the past 4 years. Dean (1952) reported low temperatures greatly affected adult parasite populations during the fall and winter of 1950. The disastrous freeze of January 30 to February 3, 1951, severely upset certain predator-prey relationships, as apparently did the January 1949 freeze. It was not until July 1951, that scale insect infestations developed to any extent, and then only in the groves interplanted with row crops, which had been treated with insecticides, particularly with DDT. The interplanted row crops provided income from land with citrus trees which had not set fruit. During the fall months of 1951, a few localized damaging infestations of chaff scale, *Parlatoria pergandii* Comst., Glover scale, *Lepidosaphes gloverii* (Pack.), and California red scale were observed. These infestations were much reduced during the following year. The drouth during the three seasons following the 1951 freeze (irrigation water being short) seemed to help cause accentuation of damage by scale on the twigs. Although these conditions seemed to favor expression of damage by scale insects, the beneficial insect-scale insect relationship improved in 1952. This was particularly evident with Glover scale and California red scale. The maximum expression of damage by chaff scale was during the fall of 1951. Scale insect damage was of very minor importance in 1954, a season during which rains brought relief from the drouth.

How to prevent or minimize economic losses from rust and Texas citrus mites without causing abnormally high scale insect populations is under study at the present time. Extremely low scale insect populations were found on those test trees not treated with any chemicals during 1954, a year apparently highly favorable for control by beneficial insects in the area. This evidence indicates that scale insects may be held to very low numbers where chemical treatments are not applied in the area. However, this condition may not be as good in the upper Valley area with chaff scale, since a limited number of beneficial insects are known to feed on this scale insect.

The factors that play a role in the biological control of citrus scale insects are presented. The scale insects are discussed in order of their relative importance in this area. More emphasis was placed on the parasitic forms. Certain parasites were introduced from cultures obtained from the Department of Biological Control, University of California, Riverside. A short section is given on certain predatory mites identified from the area.

CALIFORNIA RED SCALE.—This insect is one of the most important pests of young citrus in the area. When infested nursery trees are planted and favorable conditions prevail, they may be severely set back or actually killed by the scale. The scale is generally held to economically low population levels in older trees, except under conditions highly favorable for scale insect development.

The unnamed parasite, *Aphytis* "A", and the golden chalcid parasite, *Aphytis chrysomphali* (Mercet), were found to be the two most important natural enemies of the scale insect in this area. *Aphytis* "A" was not known to be present in the Valley before 1949 (DeBach 1951). He reported that California red scale seemed to be under control by the two parasites. From samples taken from October 1953, to October 1954, *Aphytis* "A" was found to be by far the most numerous of these two external parasites, pupal characters being used for their separation. The same predominance has also been noted in the Winter Garden district around Crystal City, Texas. California red scale is the principal scale insect pest in the latter area.

Aphytis mytilaspidis (LeB.) has also been identified from the Lower Rio Grande Valley area. However, pupal characters of samples checked indicate the parasite is of minor importance at this time.

The internal parasites, 376 *Prospaltella periciosi* Tow. and 125 *Comperiella bifasciata* How. (red scale strain), were introduced in a grove near Mercedes at Mile 11 north and Baseline Road during September 1953. Recoveries of these parasistes have not been successful to date.

Several Diaspid scale-feeding beetles are known from the area. *Chilocorus cacti* L. usually begins its largest yearly build up in late summer about the time scale insects have reached their peak population. The population continues to increase during subsequent months with adults more numerous during the fall and winter months. Following the freeze of 1951, the adult beetles were very numerous and very noticeable on defoliated trees in search of food. This species usually increases during periods of high scale populations. The Diaspid scale feeders, *Cybocephalus* sp. and *Microwesia* sp. have also been found.

CHAFF SCALE.—Chaff scale has been found to be the most numerous and widely distributed citrus scale insect in the Valley. The insect has been observed to attack twigs severely during periods of extreme drouth. Twig damage was particularly noticeable in certain groves during the fall months of 1951. Chaff scale has been found more numerous further away from the coast.

Aphytis proclia (Walk.) has been the only parasite reared from the chaff scale. Clark did not report this parasite in 1937. The parasite was apparently first reported by DeBach (1951) from the area. Dean (1952) reported the parasite to be numerous in the fall of 1951. The parasite has not been found attacking other Diaspid scale insects in this area and is considered to be the principal enemy of chaff scale. However, additional natural assistance against the scale insect would be highly desirable.

A very close association between chaff scale and a mite (undescribed genus, family Stigmaeidae) was found while checking samples from several locations. The mite has been observed feeding on first-stage scale and scale eggs. Populations of this predatory mite were found to be surprisingly high in a grove untreated with pesticides during the past 2½ years. A predatory mite, *Typhlodromus* sp. (prob. undescribed) was observed feeding upon chaff scale adult females. These *Typhlodromus* mites are probably incidental feeders on scale insects. It has been noted that where leaves are not infested with phytophagous mites, these *Typhlodromus* mites may more easily be found on scale-infested leaves.

A small black beetle, *Cybocephalus* sp., has been found to feed on chaff scale in mixed infestations of Diaspid scale, thus indicating its preference for chaff scale.

GLOVER SCALE.—The scale was observed doing quite

a lot of twig injury at three locations during the summer of 1951. Very little serious twig injury was noted in the same groves during the following year. An increase in percentage parasitism seemed to be an important contributing factor for this reduction.

The internal parasite, *Prospaltella elongata* Doz., has been found to be very important in keeping Glover scale in low numbers. Clark found the parasite to attack yellow scale in 1929 and purple scale in 1936. DeBach (1951) reported the parasite to frequently parasitize the scale, as well as *Aphytis* sp.

PURPLE SCALE, LEPIDOSAPHES BECKII (NEWMAN).—During the past 4 years, this scale was found to be somewhat of a pest in certain locations. The insect increased to rather high populations in certain locations adjacent to dusty roads and in the area nearer the coast. Purple scale was not found as prevalent in the more inland section. In this 4-year period, the scale has not been found to be as important as many of the other scale insects.

The internal parasite, *Aspidiotiphagus citrinus* (Craw), was found by Clark to attack purple scale in 1937. The parasite was taken from an elaeagnus shrub known to be infested with purple and other scale insects in October 1951. Later, this parasite and *Aphytis* sp. were found attacking purple scale on citrus. Only the male of this Aphytis was found attacking the younger stages of the scale and is believed to be *Aphytis* "A".

A colony containing 1005 *Aphytis* "X" was released (Dean 1953) in a grove south and west of Weslaco and a colony of 409 was released in a grove 2½ miles east of Mercedes in July 1952. Recoveries were not attempted until the fall of 1953. In the grove near Weslaco, many of the infested trees had been removed and the purple scale population was much reduced. Scale populations were much reduced in the other release grove also. Parasite emergence holes in older scale and *Aphytis* fecal deposits would indicate establishment, however, adult *Aphytis* "X" have not been recovered.

DeBach (1951) reported the ladybeetle, *Chilocorus cacti* L. (of which *C. confusor* Casey is a synonym) to be numerous around heavy infestations of purple and other scale insects.

Factors other than beneficial insects seem to play an important role in preventing injurious infestations from developing.

FLORIDA RED SCALE, CHRYSOMPHALUS AONIDUM (LINN.).—Heavy infestations of this scale were observed before the 1951 freeze. All trees were defoliated by the freeze with the exception of a few trees at three locations. Fruit fell a short time later. This population was greatly reduced by the removal of those portions of the tree on which the scale insect could feed. An occasional scale was found in August 1952. During the fall of 1953 and in the following year this insect where found, was not found to be parasitized. Predatory beetles were found around the small numbers that were observed.

DeBach (1951) reported immature stages of *Aphytis* sp. on this scale in 1949. Clark (1937) found the scale becoming more widespread and found *Pseudhomalopoda prima* Grlt. from the scale at only one location. Dean (1952) found this species quite common just prior to the 1951 freeze. Dean (1953) collected the parasite from an elaeagnus shrub heavily infested with the following scale insects: *Aspidiotus lantaniae* Sign., *Chrysomphalus albopictus* (Ckll.) (or close), *Aonidiella citrina* (Coq.) and purple. An opportunity to study the biological control factors of Florida red scale has not presented itself since the beginning of this study.

YELLOW SCALE, AONIDIELLA CITRINIA (COQ.).—Clark (1937) did not attempt to differentiate between California red and yellow scale in his study, but did find *Prospaltella aurantii* (How.) attacking this scale in 1929. No particular attempt was made in the present study to differentiate between the two scale insects except that samples checked were usually from that part of the tree where a predominance of California red scale would be expected. However, DeBach (1951) found *A. chrysomphali* (Mercet) and *Aphytis* "A" to commonly parasitize the scale.

Clark released a colony of the yellow scale strain of *Comperiella bifasciata* How. in the Engleman Garden's area in 1933, but the parasite was not recovered. A hurricane blew in within a month of the release. Only a small number of the older trees were found at this location on a visit in the spring of 1952 and chaff scale and Glover scale were the only scale insects found.

SOFT SCALE, COCCUS HESPERIDUM LINN.—Records at the Weslaco station indicate that infestations of soft scale were more widespread several years ago than at the present time. A greater amount of damage seemed to occur during certain years than in others. A few small trees were found to be severely damaged by the scale, although infestations were more generally found in older trees.

Dean (1953) reported soft scale infestations very high at three locations in the fall of 1951. Again in the spring of 1952, the scale increased to rather high numbers in two of these groves. Ants were tending the scale in every instance where the scale was heavy. Ants were also found to be numerous around the few light infestations found in the spring of 1954. A red-orange and black acrobatic ant, *Crematogaster laeviuscula* Mayr, and the fire ant, *Solenopsis geminata* (F.), were the common ants found around infestations. An entirely black acrobatic ant, *Crematogaster arizonensis* Whlr. (or undescribed) and *Forelius* sp. were occasionally found. Freeze damaged trees contained areas favorable for nests of the acrobatic ants. It has been noted that where ants are controlled, soft scale is not a problem.

Parasites have been generally found to effectively control soft scale when ants are controlled. Clark found *Coccophagus lecanii* (Fitch). Station records at Weslaco show this parasite as the only parasite of soft scale in this area before the present study began. Dean (1953) reported *C. lycimnia* (Walk.) as the most common parasite reared from soft scale at all locations. The species name *lycimnia* replaces *lecanii*, as the latter is a synonym. Clark also stated that *Thysanus flavopalliatus* (Ashm.) was moderately abundant (*Thysanus* parasites are hyperparasitic).

A few specimens of *Coccophagus fraternus* How., *Aneristus youngi* Grlt., *Aphycus eriococci* Timb. and *Aphycus* sp. were collected during the fall of 1951 at a location at Mile 11 North and Baseline Road. During January and February of 1952, a few specimens of *Aphycus maculipes* How., *Aphycus flavus* How. and *Thysanus fasciatus* (Grlt.) were collected. The latter parasite record was the first

for the U.S.A. *Aphycus luteolus* Timb. was released in the area in January 1954, but recovery has not been successful to date.

At a location 0.3 mile west of Sugar Road on Highway 495, *C. lycimnia* (Walk.) and *T. fasciatus* (Grlt.) were recovered during October 1951. *A. luteolus* Timb. was introduced in the grove in February 1954. Soft scale was not found a short time later and successful recovery has not been made.

Heavy soft scale infestations were found in a grove 2.3 miles north of Highway 83 on Highway 800. During September 1954, a large *Coccophagus* sp. (probably undescribed) and *A. flavus* How. were collected, and *C. lycimnia* (Walk.) was collected in the usual large numbers.

A. luteolus, *A.* "C" (China) and *Coccophagus eleaphilus* Silv. (Eritrea, Africa) were released in the grove in March 1954. *A.* "C" was recovered in September. It is interesting that the *Aphycus* spp. were not reared in large numbers in any case. The parasites are apparently restricted in effectiveness by ants which were always found when more than a few individuals of soft scale were present.

COTTONY-CUSHION SCALE, *Icerya purchasi* MASK.—Dean (1952) reported a few of the numerous instances of cottony-cushion scale infestations on pittosporum shrubs and citrus during 1951. Vedalia beetles, *Rodolia cardinalis* (Muls.) were always found when the scale was found on citrus. Where the beetles had not searched out the scale on pittosporum, the scale increased to damaging numbers and killed several shrubs. Only a few cottony-cushion scale have been noted since 1951, and in every case ants were present. The vedalia beetle is apparently the best important control for the scale in the Lower Valley area.

MISCELLANEOUS MITES.—*Cheletogenes ornatus* (C. & F.) and *Cheyletia wellsi* (Baker) have been found associated with Diaspid scale, and particularly so with chaff scale. A few specimens of *Raphignathus* sp. have been found associated with Diaspid scale. Dean (1952) found *Allothrombium* sp. to feed on Glover scale and chaff scale and noted the mite to be more numerous at certain times of the year. This mite seems to be of very minor importance in the control of scale insects.

SUMMARY.—The factors found to play a role in the biological control of the more important scale insects in the Lower Rio Grande Valley of Texas citrus belt have been presented. Climatic conditions have been found to particularly affect certain predator-prey relationships. Florida red scale was reduced by the freeze to such an extent that the pest has been slow regaining its former significance. *Aphytis* "A" (by pupal characters) was found to be the predominant parasite of California red scale. The most widespread scale insect (chaff scale) was found preyed upon by a mite (undescribed genus) particularly in untreated groves.

Soft scale and cottony-cushion scale were not found to be a problem where ants were controlled. *Aphycus* "C" (China) has been established on soft scale. *Aphycus* spp. have not been found abundant where ants were present.

Conditions favoring scale insect development and adversely affecting parasite development with certain scale insects were found to be a lack of ant control, location of trees next to dusty roads, and apparently, drift of insecticides from treated row crops.

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Some Insects Injurious to Birdsfoot Trefoil in New York[1]

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Birdsfoot trefoil, *Lotus corniculatus* L., is a leguminous plant which recently has become of importance because of its excellent possibilities as a perennial forage crop. The plant is indigenous to Europe, and is common in the region from the Mediterranean Sea northward to the Scandinavian Peninsula. It was introduced into the western hemisphere prior to 1875, and naturalized stands of the plant now are present in the United States, for the most part, in New York, Oregon, and California. However, birdsfoot trefoil was not recognized as a plant of forage value, in this country, until the mid 1920's, and extensive use of the plant was not considered until

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the development of promising cultural varieties within the past few years.

Despite the abundance of birdsfoot trefoil in Europe, reports concerning the insects detrimental to the plant are few. Kaltenbach (1894) lists several species of Homoptera, Coleoptera, Lepidoptera, and Diptera found associated with the plant. In the more recent literature, the only insect considered to be of economic importance in Europe is the gall midge, *Contarinia loti* (DeGeer). Stapel & Bovien (1943) report that the larvae of this insect feed within the ovaries of the florets of birdsfoot trefoil causing hypertrophy and loss of seed.

In this country, Montieth & Hollowell (1929) found the potato leafhopper, *Empoasca fabae* (Harr.), in damaging numbers on birdsfoot trefoil; Lincoln & Palm (1941) in a study of possible host plants of the alfalfa snout beetle, *Brachyrhinus ligustici* (L.), established that the beetle infested birdsfoot trefoil; MacDonald (1946) indicated that the clover seed chalcid, *Bruchophagus gibbus* (Boh.), occasionally is responsible for considerable loss of seed; Marshall (1951) noticed that the plant was one of the preferred hosts of the meadow spittlebug, *Philaenus leucopthalmus* (L.).

METHODS AND MATERIALS.—To obtain a more complete knowledge of the species injurious to birdsfoot trefoil, it was thought worthwhile to conduct this study. Sixty-two samples, each obtained from 100 sweeps of a net, were made over a 2-year period in 12 counties in New York, including the important birdsfoot trefoil seed growing area of Essex County. Also, mature seed pod samples, each consisting of approximately 400 pods, were collected in 13 counties of the state, and held in emergence containers to determine kinds and abundance of internal seed pests present. In addition, species thought to be injurious were placed in small cylindrical wire screen cages, over various stages of birdsfoot trefoil, to obtain qualitative information concerning the nature of the damage. Finally, many hours were spent in the field observing the insect species on the plant to further add to the data concerning their detrimental habits.

DISCUSSION.—The following is a list of insects collected from birdsfoot trefoil. These data are derived from a combined analysis of net sweep information, cage experiments and field observations. Any insects which may have been collected in net sweeps, but were not seen during the frequent field studies to be a more or less constant inhabitant of birdsfoot trefoil stands, i.e. forms merely using the trefoil as temporary cover, have been excluded from the list. Those species which were found to be definitely destructive are so designated by an asterisk.

HOMOPTERA

CERCOPIDAE
Philaenus leucophthalmus (L.)*
CICADELLIDAE
Aphrodes costata (Panzer)
Scaphytopius sp. prob. *acutus* (Say)
Athysannus argentatus (F.)
Agallia quadripunctata (Prov.)
Empoasca fabae (Harr.)*
Aceratagallia sanguinolenta (Prov.)
Macrosteles fascifrons (Stal.)
APHIDIDAE
Macrosiphum pisi (Harr.)

HEMIPTERA

MIRIDAE
Adelphocoris lineolatus (Goeze)*
Adelphocoris rapidus (Say)*
Plagiognathus politus Uhler
Plagiognathus chrysanthemi (Wolff)
Lygus lineolaris (P. de B.)*
ANTHOCORIDAE
Orius insidiosus (Say)
NABIDAE
Nabis ferus (L.)
Nabis subcoleoptratus (Kirby)
PENTATOMIDAE
Euschistus variolaris (P. de B.)*

ORTHOPTERA

LOCUSTIDAE[a]
Melanoplus femur-rubrum (Deg.)*
Melanoplus bivittatus (Say)*
Melanoplus differentialis (Thos.)*
GRYLLIDAE
Nemobius fasciatus fasciatus (Deg.)*
Acheta assimilis (F.)*

THYSANOPTERA

THRIPIDAE
Frankliniella tritici (Fitch)*
AEOLOTRIPIDAE
Aeolothrips fasciatus (L.)

COLEOPTERA

NITIDULIDAE
Brachypterolus pulicarius L.*
CURCULIONIDAE
Brachyrhinus ligustici (L.)*
COCCINELLIDAE
Adelia bipunctata (L.)

LEPIDOPTERA

TORTRICIDAE
Archips rosaceana (Harr.)*
Sparganothis sulphurana (F.)*
Tortrix pallorana (Robn.)*
Tortrix velutinana (Wlkr.)*
NOCTUIDAE
Plathypena scabra (F.)*

DIPTERA

LARVAEVORIDAE
Actia interrupta Curr.
BOMBYLLIDAE
Villa sp.
SYRPHIDAE
Mesograpta marginata (Say)
Sphaerophoria cylindrica (Say)
Sphaerophoria menthastri (L.)

HYMENOPTERA

EURYTOMIDAE
Bruchophagus gibbus (Boh.)*
EUPELMIDAE
Eupelmella vesicularis (Retz.)
PTEROMALIDAE
Amblymerus sp.
EULOPHIDAE
Tetrastichus bruchophagi Gah.
ICHNEUMONIDAE
Glypta simplicipes Cress.

[a] Partial list.

Glypta rufofasciata Cress.
Itoplectis conquistor (Say)

APIDAE
Apis mellifera L.
Bombus vagans Sm.
Bombus fervidus F.

HALICTIDAE
Augochlora stirata Prov.
Halictus pilosus Sm.
Halictus sp.

ADRENIDAE
Andrena wilkella Kby.

MEGACHILIDAE
Hoplitis producta (Cress.)
Osmia coerulescens (L.)
Osmia inspergens Lov. & Ckll.
Megachile relativa Cress.
Megachile latimanus Say
Megachile brevis Say
Megachile mendica Cress

As is apparent from the list, injurious species, representing all the major orders except the Diptera, were found. Of all the orders, homopterous insects outnumbered all others present. The most abundant species was the meadow spittlebug. The damage caused by the nymphs of this insect to birdsfoot trefoil was similar to that found on other host plants. The young fed on the stems and petioles of the plant withdrawing the juices, which resulted in stunting and rosetting. Marshall (1951) found that the number of immature spittlebugs on birdsfoot trefoil consistently exceeded the number found on other leguminous hosts, and during this investigation it was noted that when the bugs become mature they prefer to remain on these plants. This was evidenced by the fact that as high as 8,000 spittlebug adults were collected per 100 net sweeps in some fields investigated. The wilting and blighting of birdsfoot trefoil terminal growth was common in most fields, and it was thought that, in areas having high spittlebug populations, perhaps some of the blighting was caused by heavy adult feeding. Cage experiments with the adult indicated that the feeding of 12 insects per shoot over a 4-day period produced wilting and dying of the terminals. Spittlebug adults were seen to feed on the seed pods of birdsfoot trefoil. However, the ability of the insects to damage the seed of the crop was not definitely established.

The pea aphid, *Macrosiphum pisi* (Harr.), was found in moderate numbers in fields of birdsfoot trefoil in the spring. A maximum of 300 individuals per 100 net sweeps were obtained, which is a lower population than one would expect on red clover or alfalfa. Adults and nymphs fed mainly on the stem, petioles, and leaves, and they also were seen in limited numbers feeding on the seed pods. No noticeable injury to the plant was apparent, but it is expected that a certain loss of plant vigor resulted with maximum infestation.

The potato leafhopper was present in fields of birdsfoot trefoil from early June to early September. Observations concerning the damaging effects of the insect closely agree with the findings of Montieth & Hollowell (1929). Adult and nymphal feeding resulted in chloronemia and anthocyanescence of the center and apical portion of the leaflets, and severe leaflet tip burn. Heavy leafhopper injury was noticed during mid-July in a few fields which had not been mowed in the spring. However, in the majority of stands, damage by the leafhopper seldom reached severe enough proportions to be readily visible.

The clover leafhopper, *Aceratagallia sanguinolenta* (Prov.), the six-spotted leafhopper, *Macrosteles fascifrons* (Stal.), and other leafhoppers occurred in association with birdsfoot trefoil. However, these forms were present in limited numbers, and no apparent injury was found to be caused by these species.

Insects of the order Hemiptera were next in abundance. Of these, large numbers of plant bugs were frequently collected. During the course of this investigation, the alfalfa plant bug, *Adelphocoris lineolatus* (Goeze), and the rapid plant bug, *Adelphocoris rapidus* Say, proved to be two serious pests of birdsfoot trefoil. Adults and nymphs fed upon the mature floret buds and the blossoms. Their feeding activities resulted in a blasting of these reproductive parts. Also, adult females oviposited within the stem of the plant, inducing wilt and blighting of the terminal portions. In addition, the caging of adults and nymphs on the seed pods produced a high percentage of shriveled seed. While both these plant bugs are equally capable of causing damage, net sweeps and field inspection indicated that in New York the alfalfa plant bug far outnumbers the rapid plant bug.

Damage of a similar nature to that produced by the alfalfa plant bug and rapid plant bug was inflicted by the tarnished plant bug, *Lygus lineolaris* (P. de B.). This species, however, preferred to feed on the newly formed and partially mature floret buds of birdsfoot trefoil, causing the buds to blast; little damage was caused to the older buds or blossoms, and no oviposition in the stem was apparent. Later in the season, the bugs also fed on the green seed pods; they pierced the valves and sucked the juices from the developing seeds reducing them to shriveled seed coats.

In addition to the seed damage caused by the feeding of Miridae, seed injury was produced by the stink bug, *Euschistus variolaris* (P. de B.). Adult bugs were collected from seed pods toward the latter part of July in northern New York. These insects proved to be active, destructive feeders. However, they were present in limited numbers.

Other Hemiptera were found to feed on birdsfoot trefoil, but as with many of the Homoptera, only a few were present, and they appear at this time to be harmless to the growth and development of the plant.

Many species of Orthoptera were collected from birdsfoot trefoil. The nymphs and adults of the red-legged grasshopper, *Melanoplus femur-rubrum* (Deg.), and the two-striped grasshopper, *Melanoplus bivittatus* (Say), were the more dominant forms. These hoppers chewed irregular holes along the margins of leaflets, or occasionally consumed entire leaves. The green seed pods were also severely notched and chewed. However, relatively few individuals of each grasshopper species were present, and the damage, therefore, was negligible.

Extensive field cricket damage to birdsfoot trefoil seed pods was noted in early September in Essex County. The valves of brown seed pods, while still on the plant, were shredded along the sutures, and the seed removed. Both *Acheta assimilus* (F.), and *Nemobius fasciatus fasciatus* (Deg.), were collected from the damaged area, and cage tests with these species produced similar injury.

Unfortunately, however, both species were placed within each of the test cages, and, therefore, it is not possible to evaluate the relative importance of the two insects.

The order Thysanoptera was represented by a single species of plant-feeding thrips, the flower thrips, *Frankliniella tritici* (Fitch). These thrips were collected in moderate numbers from the florets of birdsfoot trefoil in early June, in north central New York, when the plants were just starting to bloom, and they continued to be present to a lesser extent throughout the growing season. Florets examined for thrips damage revealed considerable feeding on those portions of the wing petals which are concealed by the standard. This whitish pitting of the normally yellow petals appeared to have little effect on the normal development of the florets and seed. In association with the flower thrips, a few predaceous *Aeolothrips fasciatus* (L.) were usually present.

Of the Coleoptera collected, a small nitidulid beetle, *Brachypterolus pulicarius* L., was quite abundant during the initial blooming period of the birdsfoot trefoil. As with the thrips, it also fed within the blossoms. However, it was seen to attack unexpanded florets, as well as those fully opened. With closed florets, the beetles chewed their way through the corolla, usually making a ragged hole in the keel, and made their way to the stamens. Here they fed on the pollen, usually severed the anthers, and occasionally damaged the pistil. In feeding on the reproductive organs of the floret, bits of pollen and frass were scattered about within the corolla. Individuals caged on florets demonstrated their ability to impair proper fertilization, which resulted in lack of seed pod formation.

An examination in Oswego County confirmed the findings of Lincoln & Palm (1941) that birdsfoot trefoil may act as a host for the alfalfa snout beetle. The small larvae consumed the root hairs, while the more mature larvae fed externally on the larger roots, grooving them deeply. The adults also fed on the foliage. This snout beetle is limited in its range within the state, and is therefore of minor importance in most stands.

While the Coleoptera were represented by only two destructive species, five species of Lepidoptera were found to cause injury to birdsfoot trefoil. Several larvae of the family Tortricidae webbed and rolled the terminal portions of the plant together forming a shelter within which they fed. The growing tip of the shoot was severed by the larvae, and was found occasionally uneaten, dried, and webbed externally to the top or side of the structure. Twisting of the stems usually resulted after a period of time because of the confinement of the upper leaves. As the larvae matured they moved to adjacent plants forming similar living quarters having adequate space. In one of the fields under observation, leaf rollers averaged more than one per square foot. Specimens reared through to the adult stage were identified as the oblique-banded leaf roller (*Archips rosaceana* (Harr.), *Sparganothis sulphurana* (F.), *Tortrix pallorana* (Robn.), and *Tortrix velutinana* (Wlkr.). Approximately 50% of the adults were identified as the oblique-banded leaf roller. The ichneumonids, *Glypta simplicipes* Cress., *Glypta rufofasciata Cress.*, and *Itoplectis conquistor* (Say), and one dipterous parasite *Actia interrupta* Curr., were reared from the larvae of this more numerous species.

The green clover worm, *Plathypena scabra* (F.), was collected in small numbers on birdsfoot trefoil. The larvae were seen to feed on the foliage incising the leaves. In addition, they were found consuming green seed pods.

The only destructive insect of the order Hymenoptera was found to be the clover seed chalcid, which has long been recognized as a pest of red clover and alfalfa seed. As with the other host plants, the female clover seed chalcid was seen to lay her eggs within the developing seeds of the maturing seed pod. Oviposition was found to take place approximately 10 days after pollination of the floret. After hatching, the larvae fed on the young cotelydons until only the seed coat remained. Evidence of the internal feeding manifested itself externally in lighter coloration and a slight loss of plumpness of the mature seed. The majority of the adults usually emerged after a short pupal period within the seed, to mate almost immediately, and start a new generation. The amount of infestation, based on adults emerging from seed pods collected in New York, reached a high of approximately 17 per cent, with a 1 to 2 per cent infestation being found in most fields.

Several parasites of the clover seed chalcid also were collected from the emergence containers. The most abundant of these was *Habrocytus medicagnis* Gah. The species *Tetrastichus bruchophagi* Gah., *Eupelmella vesicularis* (Retz.), and *Amblymerus* sp. also were present.

SUMMARY.—To supplement the limited information concerning the injurious insects associated with birdsfoot trefoil, a study of the complex was undertaken through the use of net sweep sampling, caging, collection of seed pods, and field observation. Injurious species, representing all the major orders except the Diptera, were found. Homopterous insects were the most abundant, particularly the meadow spittlebug, which caused rosetting and blighting of the plant terminals. Of the Hemiptera found, the alfalfa plant bug appeared to be the most destructive species because of its abundance, and its ability to produce blighted plant terminals, blasted floret buds and blossoms, and shriveled seed. Many species of Orthoptera were seen to feed on the foliage and seed pods of birdsfoot trefoil; grasshoppers consumed leaflets and green seed pods, while field crickets destroyed the mature brown seed pods. Beetles, of the family Nitidulidae, were found to damage the reproductive parts of the florets. Four species of Lepidoptera injured the terminal growth of the plant by webbing the upper leaves together, and feeding within these shelters. In most fields of birdsfoot trefoil in New York, the clover seed chalcid was found to destroy 1 to 2 per cent of the seed.

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The Ecological Approach to Management of Insect Populations[1]

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The basic principle underlying insect control involves the management of at least some segment of the population in question. This is true regardless of the methods employed, as in every case the attempt is made to adjust population numbers. Whether it is best to do this directly, by the application of insecticides, or indirectly, through the manipulation of the habitat in which the insect lives, can only be determined by the nature of the problems involved. For example, it became evident some years ago that we could not satisfactorily and economically control rangeland-infesting grasshoppers by the use of insecticides alone—even if highly toxic and efficient materials became available—because of the vast areas involved and the low real or rental value of a large proportion of these lands. After considering and exploring many phases of the problem, the most reasonable avenue of approach appeared to be one aimed at preventing, in so far as possible, large population build-ups and supplementing the prevention program with chemical control in areas or under circumstances where prevention was either impractical or impossible. It will be seen that this approach involves the management of insect populations in their natural habitats and is dependent on the manipulation of those factors in the habitat which contribute to the occurrence of high population densities.

If given an optimum environment in all respects, indefinite increase in populations would be possible. However, environmental conditions of the globe vary from point to point and are probably in no two places exactly alike, nor do they remain constant in time. From this it follows that at a given moment, in a given area, the precise environmental complex which would constitute the optimum for a particular species will be found at relatively few points. This is generally proposed as the real reason why organisms do not often increase to the point of becoming plagues. The fact that the universe is composed of a variety of specifically different and interacting animals implies a limited possibility of existence for any one of them. I am inclined to agree with the statement of Thompson (1939) that a condition under which an organism cannot realize all its potentialities is the normal, and high populations are the abnormal.

We usually assume that the environmental relationships are in some way responsible for animal numbers. Since it is generally agreed that balance, in the sense of stability or oscillation about a mean, is at least approximately realized in nature, we know that something has to act as a governor to uninhibited increase in animal populations. We have ample evidence that the potential for unlimited increase is ever present, especially in insects. There are two elements to be considered: first, the organism, with its inherent tendency to survive and to reproduce without limitation; and second, the environment, with its limited capacity for satisfying these tendencies.

The management of at least semi-natural animal populations has received its greatest application in the field of fish and wildlife management, where certain practices are recommended in order to maintain the highest sustained and efficient animal population. It is interesting to note that entomologists often recommend essentially the same practices in order to decrease an insect population. Obviously the use of the same means to obtain opposite ends could be justified only if adequate data were available to show that a "principle" developed from studies on vertebrate behavior had no application to insect behavior. Unfortunately, as far as is known, no such data are available. Much of the insect data that are available do not lend themselves to an analysis comparable to that which has been carried out for the higher animals. Until such time as quantitative data are at our disposal which will disprove for insect species the principles developed through studies on other animals, a working hypothesis for insect populations of necessity must be based on the knowledge derived from studies on a wide variety of animal species. To illustrate this point it is proposed to review some of the more pertinent principles on populations control developed through studies on other animals and to see how closely these principles apply to observations and data gathered on one group of insects, viz., grasshopper species infesting rangelands, on which considerable research has been done.

To review these principles, it is necessary to understand the terms and the meanings implied in their use by different workers. Although this might seem elementary, we have to consider but two terms, "habitat" and "environment," which are basic to the science of ecology, to realize the diversity in usage which exists. These two terms are apparently somewhat elusive and difficult to define concretely. The literature reveals that most authors, when reporting on individual research, define these terms with specific reference to their particular problem. If the paper is of a general ecological nature, the terms are defined in their broadest sense. Odum (1953), for example, defines the habitat of an organism as the place where the organism normally lives or as the place occupied by an entire community. On the other hand, Dowdeswell (1952) divides habitats into four broad classifications; namely, terrestrial, marine, estuarian, and fresh water. His interpretation of a habitat is therefore geographical in nature. Glen (1954) lays some stress on the primary habitat unit as being closely linked with the primary taxonomic unit; thus, his usage is with reference to the home of the individual species. In a recent publication Elton (1954) has introduced the term "arena" to denote a clearly recognizable structural unit defining the place where a mixed population of species is engaged in the struggle for survival. In this discussion the term

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"range" will be used to designate the whole area occupied by a particular species and the term "preferred habitat" as the area within the range in which the organism prefers to remain or the area it will seek out if given a choice. The range, as well as the specific habitat, is then limited by the preference of the animals provided they are not excluded by natural barriers. The range and habitat preference may be expanded by adaptations within the animal which normally take place slowly and are frequently overlooked.

The term "environment" is still more difficult to define, except in a very general way. Combining the main thoughts expressed by many authors, the environment of an animal could be defined as a collective term including all the factors, both living and non-living, which may have an influence on the behavior of the animal. It would then be made up of all factors both biotic and physical. Along with the physical and chemical aspects, the animals themselves are just as much a part of that environment as are the other animals and plants that surround them. Many of us are too prone to forget or disregard this fact and to look for and expect single casual factors, such as temperature, humidity, food, etc., in an animal's external environment to provide the answers to our problems. If we are going to accept the thesis that animals are dependent on their environment, then it would seem that we must accept the thesis that no one factor can constitute this environment. The complete environment with all its interrelated and modifying characteristics can and does involve considerably more than weather, climate, parasites and predators, competition, food, shelter, etc., either by themselves or as modifiers of each other.

If we accept the thesis that the distribution of a species is limited by its habitat tolerance or preference, we would not expect to find any species uniformly distributed throughout its geographic range unless the habitat were uniform over all. The more probable situation would be to find the species populating limited areas within its range at different degrees of population density. The reaction of a species to the entire environmental complex within the habitat would be reflected by the degree of preference (in terms of relative numbers present) exhibited by them for the conditions which exist.

As the population within the habitat increases a toleration factor, the importance of which is determined by the forbearance the individual members of the species have for each other, becomes operative. This factor may function as a density dependent one or it may express itself in a physiological reaction which is more or less independent of density, as Errington (1943) found to be the case with the muskrat. When the point of intolerance is reached, certain individuals of the population are forced out of their preferred habitat into other habitats which are less favorable. A determination of habitat preference would be somewhat simplified if it were not for complications induced by the toleration factor which is the underlying force responsible for territorial behavior in animals. As far as is known now, most if not all animals exhibit territorial behavior to varying degrees during some period in their life history. This behavior is recurring and is apparently induced through a change in the physiology of the animal and is most often expressed during the breeding season. The work of Hochbaum (1944) on waterfowl demonstrates rather conclusively that the habitat requirements for these animals may change considerably during the breeding season. At this time the degree of tolerance between individuals of the population decreases to the point where the population must extend its area of occupancy far beyond that required at other times. These new areas of occupance—or territories, as Hochbaum calls them—are in reality specialized pieces of terrain in which certain definite components must exist together before they are suitable for habitation and reproductive activities. If the available habitat is not extensive enough to accommodate all the individuals or does not have all the necessary components, which in many instances may be the case, it becomes rather obvious that territorial behavior could be the weak link in the chain that determines population levels.

When the activities of grasshoppers are considered, it is not too surprising to find that certain species at least appear to have essentially the same behavior patterns as those described above for vertebrate animals. There are many references in the literature to observations on grasshopper behavior which could be interpreted as both an exhibition of varying degrees of tolerance and territorial behavior. Before discussing these phenomena, however, it might be well to cite some of the Montana observations on grasshopper habitat preference.

Among all the range grasshopper species which have been observed, one essential factor in their selected habitat is the presence of their preferred host plant or plants. Another factor which appears to be of considerable importance is the physical state of the vegetation; that is, whether it is dense or sparse, tall or short, as well as the presence or absence of small areas completely nude of vegetation. These physical requirements differ considerably between grasshopper species. A high degree of specificity in the selection of its particular habitat is apparently shown by each species. This does not imply, however, that all selected habitats are populated to the same degree and that these habitats will remain populated to the same extent throughout the season. In fact, quite the contrary appears to be the general rule. The underlying reasons behind these differences and changes are probably involved in an interaction of factors other than food plants, the physical state of the vegetation, etc. It is not uncommon at any time to find some individuals in a habitat that appears marginal or even unfavorable for the species, judged on the basis of areas they most frequently occupy. These may be individuals who were forced out of the more favorable places due to strife resulting from too high a population density, while at other times they may possibly be the victims of territorial behavior.

While generally speaking most grasshopper species exhibit gregarious tendencies, evidence of intolerance between individuals has been noted at some stage among all of the grasshopper species studied. In some cases the degree of intolerance appears to increase with population density and is especially evident during the mating and egg-laying season. It is exhibited toward members of different species as well as among those of the same species. To what extent this highly excitable reaction, which

results in strife among the members, contributes to dispersion or the forcing of individuals out of their preferred habitat is difficult to measure in a quantitative manner but it undoubtedly is one of the determining factors in their distribution.

A type of territorial behavior has been noted in at least one of the range species which have been studied. In this case the reaction was quite obvious. The species leaves the short grass habitat in which it developed to occupy areas of tall bunch grass where an opportunity is afforded it to remain concealed most of the time from other members of the species. There appears to be an obvious effort on its part to remain hidden or out of sight from other individuals. This results in the species having to occupy a much larger, rather specific type of territory when such is available. The reaction is confined only to the mating and oviposition period. While this phenomenon has not been observed specifically in more than one species, it does not rule out the possibility that it may be a characteristic of all or many species. More exacting observations and measurements will be necessary before this point can be settled. The interspecies relationships referred to above are good examples of the importance of the animals themselves as an operating ecological factor.

Earlier in this discussion reference was made to the fact that the grasshopper population density varied considerably between selected habitats. Measurements of vegetative type and density as well as other physical factors revealed differences which might account in part for the observed variance in grasshopper numbers. At present it is not possible to assign a limiting importance to any single factor or combination of factors. Additional research and the consideration of stresses not presently being measured or even understood will be necessary for a true understanding of the population differences which exist. Errington's concept of a security threshold which may vary considerably from one habitat to another also offers a possible explanation for the observed differences in population density. This concept of Errington's is best understood by further reference to his work on the muskrat. Consider first the muskrat situation during the winter months. A typical habitat is a lake or a marsh which contains adequate food to carry a particular population through until spring. During this period the marsh is frozen over, so with tolerance at a maximum and an adequate food supply it is not necessary for any individuals to leave their secure winter quarters and thus expose themselves to predation or other mortality factors of an insecure environment. The result is that the entire population can live harmoniously in this rather restricted area with comparative safety. When spring comes the factors of intolerance and territorial behavior become operative with the result that many of the muskrats are now forced to leave the safety of the home marsh to seek quarters elsewhere. During this period of exposure they fall prey to many of their natural enemies as well as to other mortality factors with the result that there is a heavy loss among these so-called migrants. Since most if not all of the marshes lakes, etc., within the area are populated during the winter in excess of what will be tolerated during the breeding season, those migrants which do not fall prey to their natural enemies, etc., in most cases must select marginally secure or even submarginal territories in which their chances of survival are greatly diminished. In other words, they must select a habitat whose security threshold is much lower.

Since the security threshold of a particular habitat is so dependent upon the presence of the right type of food and shelter, it can never be looked upon as being constant for a particular habitat. Again referring to the muskrat, if the water level of the marsh were lowered through drainage or as a result of drought conditions, the security threshold for that particular marsh or portion of the marsh would be lowered as each successive sector lost its specific set of characteristics. These characteristics provide necessary conditions for security for that segment of the population. As the extent of secure habitat is diminished, more of the inhabitants would be forced to occupy marginal or submarginal habitats. The number of individuals a habitat could support would then vary, either up or down, as a result of any change in the habitat. These changes in a habitat could also be brought about by the activities of the individuals themselves. The threshold of security, then, defines the upper limit of population density a particular habitat is capable of maintaining in a relatively secure state.

Before attempting to draw further analogies between muskrat and grasshopper behavior, a typical behavior pattern which would with minor modifications fit most of our rangeland grasshopper species should be described. At a period immediately following hatching it is found that most early instar nymphs show a definite tendency to remain congregated in or near their hatching beds. This gregarious characteristic is more pronounced in some species than in others but is evident to some extent in all of them. As they develop through successive instars, the attraction for each other appears to lessen and is gradually replaced by a tendency to disperse. This tendency is more readily observed if the population density is high and is expressed between members of the same species as well as those of other species. These movements show no evidence of orientation and the new areas of occupancy are limited, at least in part, by the presence of suitable host plants. When the adult stage is reached the dispersion is such as to suggest an attitude of intolerance and results in many individuals now having to expose themselves through being forced out of the protection afforded by the original habitat. During this period if their preferred-food supply becomes exhausted some species will wander into other areas in search of preferred host plants, while others will remain in their original habitat and feed on any dry plant material, trash, or other organic matter that is available. When mating starts, some species exhibit a very high degree of intolerance resulting in further dispersion, while others seek an entirely different habitat, and still others have a tendency to congregate again. In cases of very high population density many individuals are forced to occupy places which they may not have selected otherwise, thus exposing them to additional mortality factors.

Thus it will be seen that the general behavior pattern for at least some grasshopper species resembles in many ways that described by Errington for the muskrat. This being the case, one would expect that the principles of

natural population control as described by the above author for the muskrat might also apply with some modifications to the grasshopper. For instance, the concept of a security threshold could explain why some preferred habitats, commonly referred to as "hot spots," could always maintain a higher population density than surrounding habitats of a somewhat different nature. It could well be that these "hot spots" include in their make-up some physical factor or factors which would result in their having a higher threshold of security. Viewed in this light they would not constitute a menace, as has often been proposed, through their acting as a focal point from which outbreaks develop. During the so-called outbreak stage of Montana rangeland grasshopper species, the various habitats in which they occur are never populated to the same density level. It can be shown that the scarcity of host plants is not in itself a limiting factor for in some instances small populations occur where numerous host plants are present, while in immediately adjoining areas where few host plants prevail high grasshopper populations are frequently encountered. There is always the possibility of those 'hoppers which are forced out of a preferred habitat creating by their own activities a habitat which could remain suitable at least for a short period of time.

The Montana studies have pointed out that the physical nature of a habitat plays a very decided role in determining the ultimate grasshopper population within that particular habitat. It is questionable that an increased rate of reproduction could change the permanent population level within a habitat which has a particular security threshold level. In view of the importance of the habitat, it seems reasonable to assume that, at least with some grasshopper species, the chance to survive is of more significance than the ability to produce large numbers in determining the ultimate population level. This point is well expressed by Errington who says:

"One widely held apparent misconception has to do with muskrat fecundity as a safeguard against undue depletion of the species through predation. It is true that unusually severe losses of young may be partially offset by prolongation of breeding, but the Iowa data, as interpreted, do not support the thesis that the muskrat has to be as prolific as it is to maintain itself in the face of its many enemies and that, the more prolific a race of muskrats may be, the better it is adjusted to withstand predator pressure. It seems highly probable that the more prolific a race is, the more predation it will draw, which is a different thing from keeping ahead of its enemies through sheer rapidity of increase. Here again the old 'fallacies of misplaced concreteness' add their confusion, to perceive which we need but remind ourselves of the nature of predation upon muskrats. Patently, the self-limiting mechanisms behind muskrat population phenomena operate only partially through reduction in number of individuals born per breeding female and then largely during periods of emergency or in years marked by either extremely low or extremely high muskrat densities. For the majority of low to moderate density muskrat populations observed in the course of the Iowa researches, recovery during 'normal' breeding seasons tended to be more or less predetermined by intraspecific and habitat factors, irrespective of the average numbers of young born per female and the populations status of such predators as minks. The theoretical advantages of many or large litters are in good measure nullified by greater wastage, through intraspecific strife if through no other medium. Conversely, the theoretical disadvantages of few or smaller litters become counter-balanced by superior chances for survival of the young that are born, with resultant diminishing in manifestations of predation."

Whether or not every facet of Errington's theories on muskrat population dynamics holds true for all grasshopper species can be proven only through studies on the nature of the interrelationships which exist between the grasshopper, its habitat, and its natural enemies. There is no easy solution to this problem as population data alone are meaningless unless they can be definitely associated with the habitat and the specific activities of both the grasshopper and that of its parasites and predators.

On purely theoretical grounds it can be shown that it is all to the advantage of the parasite or predator to have its host develop under the most favorable condition. Since the host furnishes the complete environment of a parasite during at least some stage in its development, the relationship between the two must be such that the parasite does not over-exert its influence on the host. It is reasonable to assume that the most favorable relationship between the parasite and the host developed as a result of parallel evolution and that the host controls the population of the parasite by providing an essential environment for it.

It can also be shown on theoretical grounds that if a predator or all stages of a parasite and the host inhabit the same identical habitat a favorable population balance could not be maintained, as under these conditions the host—and hence the predator or parasite—would be eliminated completely. The most favorable situation could be one in which the host and its parasite or predator inhabit essentially different but intermingling habitats. Under these circumstances the surplus from the host habitat, when forced out, would automatically invade the parasite or predator habitat where it would be subjected to their attack. Since the surplus individuals would be disposed of eventually by one means or another, the parasite or predator is not actually affecting the basically secure population but is merely hastening the disposal of those individuals who are in excess of what the habitat can securely maintain. If the host through increased numbers and its own activities could create a habitat suitable to its enemy, then the enemy might be expected to have an effect on the secure host population. If, on the other hand, during the period of intolerance the surplus population was not disposed of and it was allowed to return to the original habitat, an excessively dense population might result. Under these circumstances the parasites and predators can be looked upon as beneficial rather than detrimental to the host, for without this check the host could eventually destroy its own habitat. There is considerable evidence to substantiate the belief that the parasite or predator has its own selective habitat and that it is through its wanderings within this habitat that it encounters its host or prey, as the case might be. Without a knowledge of the true relationships of both the host and the enemy to their environment as well as an understanding of the interrelationships between the two populations, there is no basis for postulating any effects, either beneficial or detrimental, of parasites or predators on the host populations.

From the available data one can conclude that with grasshoppers the degree of both parasitism and predation is highly variable within any given area. No particular correlation can be demonstrated between the density of either parasite or predator and that of the grasshopper, indicating that this density factor alone is not the determining one in parasitism or predation. It is not uncommon for the implication to be made, from reports of heavy parasitism or predation in certain areas, that parasites or predators are bringing a population of grasshoppers under control. In these cases no attempt has been made to associate the sudden high incidence of parasitism or predation with any corresponding change in the host habitat. It is merely assumed that the above situation developed as a result of a rapid increase in the parasite or predator with complete disregard for the habitat factor.

The rapidity with which a habitat can change has been demonstrated on many occasions. Such factors as weather, the activities of man and domestic or wild animals, as well as the activities of the grasshoppers themselves, can bring about a rather abrupt and sometimes almost complete change in a habitat within a relatively short period of time. These changes are most certainly reflected in one way or another in the behavior of the resident grasshoppers. If the changes result in a lowering of the security threshold of the habitat, the same enemy population could account for a much higher degree of parasitism or predation; or, if the changes were more favorable to an increased rate of reproduction of the host, a greater surplus would develop. In either case the habitat would be the modifying agent and not the parasite or predator. With our present knowledge it appears reasonable to assume that the major role played by parasites and predators is through their disposing of part of the surplus population over and above that which can be securely maintained by the particular habitat. The surplus population will be made up of those individuals who were forced to invade the insecure habitats, which makes them more vulnerable to all mortality factors—not only to parasites and predators. How many become surplus will be determined by the habitat, the rate of reproduction, and the effect of other forces such as weather, food, etc., on early instar mortality.

Theoretically, if uninhibited, a population will increase exponentially. Actually, this situation is never realized as all population curves are of the typical S-shaped type, indicating that a physiological effect sets in at some particular population density which causes a decided decrease in the rate of reproduction. This phenomenon is discussed in some detail by Clarke (1954) and appears to hold true for all classes of animals. The density level at which a population is held will have an effect on its rate of reproduction, provided that no other environmental factor becomes limiting. The removal of a portion of the population may then affect the rate of reproduction in one of two ways: it may depress an already low reproductive rate, or it may accelerate the rate of reproduction in a situation where the density had already exceeded that of maximum reproductive rate. These principles are recognized and employed by wildlife biologists in their attempts to maintain the highest sustained efficient populations at all times. These biologists have come to recognize the operation of two important principles in the populations with which they are dealing: first, that the nature of the specific habitat determines the number of animals it can securely maintain; and second, that if the surplus population is not harvested each year a point may be reached where reproduction rate decreases. In a naturally balanced situation these surplus animals would be disposed of through such factors as predators, starvation, etc.; under a management program they should be harvested by sportsmen.

In the case of the grasshoppers the excess population may be disposed of either through natural or artificial control or through a combination of both. Many theories have been advanced to explain the mode of operation in natural control factors. These are discussed by Solomon (1949) who says:

> "The earlier theories looked to natural enemies as the main agents of control. Somewhat later theories gave preponderance to physical factors. Control has been attributed exclusively to competition between natural enemies, or more broadly, to density-dependent mortality. Among theories of periodic fluctuations, many ascribe control to some aspect of overpopulation of the environment, while mathematical theories envisage control due primarily to natural enemies. Certain comprehensive theories lay emphasis on the complexity and essential interconnectedness of the population-environment system, and hold that the particular causes of control vary with the circumstances."

Our western grassland ranges are almost continually undergoing changes in plant density and composition as a result of weather and land management practices. In a recent publication Scharff (1954) pointed out the possible effect of these changes on the reproductive potential of the grasshopper species *Melanoplus mexicanus*. That the diet may have a pronounced effect on the rate of development and reproduction among the lower organisms has been amply demonstrated. By varying one vital component in the diet of many organisms, their rate of development and reproduction can be made to vary from a low in which they are able only to maintain themselves to a high where maximum growth and reproduction are obtained.

The presence or absence of a particular species of plant at a given time in the life cycle of grasshoppers may be an important factor in determining developmental and reproductive rates. It is possible also that the nutritional requirements change as the grasshopper develops. This is based on the observation that food preferences often change with some particular instar (Anderson & Wright 1952). Along with the possible changes in requirements of the grasshopper, there are several ways in which the dietary component of the environment may vary. It has been observed that the appearance of certain annual plants does not necessarily coincide with the same stage of development of the grasshopper from one year to the next. Lack of adequate moisture at a given time may result in the absence of a particular plant for that season. These dietary factors may account for the heavy first-instar mortality so often reported. It is usually assumed that this heavy mortality is due to the direct effect of weather while in reality the weather effect may be an

indirect one through its effect in bringing about a change in the plant complex.

The Montana observations have tended to conform to the already established fact that the greatest changes in vegetation, both qualitative and quantitative, are brought about by a combination of weather and land management practices. While weather is beyond the control of man, land management is not. Almost without exception the Montana studies have found grasshoppers in high numbers to be associated with such practices as excessive grazing by livestock, allowing cultivated land to revert, or to a combination of heavy grazing and dry weather. Under extreme conditions weather alone can bring about these changes, but the usual situation involves a certain amount of help by man. One general rule that can be applied to these changes in habitat is that in recessions the order is from perennials to biennials to annuals, while in successions the reverse is true. Certain species of grasshoppers are associated with each stage in recession and succession.

As long ago as 1928 Uvarov suggested an ecological approach to the control of the Moroccan locust. He based his arguments on the theory that this locust could breed up to outbreak numbers only within rather specific habitats and that a destruction of these habitats would at least help to alleviate the problem. Several years later (1933) he published the results of further studies on this subject from which he concluded that ". . . field studies in Western Anatolia have provided sufficient proof that reservations from which locust outbreaks start and spread over wide regions are not numerous and are well defined. Indeed, the reservations proved to be concentrated within strictly defined geographical, vegetational, and climatic zones, and moreover very narrowly localized, representing often only small 'islands' which can be identified by their soil and vegetation and accurately mapped out." Uvarov then suggests that control measures be applied to these reservations before the locusts disperse and concludes his paper with the statement, "There is, indeed, no justification for continuing the wasteful policy of controlling the Moroccan locust on a defensive basis, and antilocust organisations would be well advised to concentrate on the study and control of reservations."

As it can now be seen, Uvarov's original idea can be projected further to include the type of management which, in the first place, would minimize the development of these favorable reservations or habitats. This would include both the altering of the physical nature of the habitat and the elimination, in so far as possible, through proper land management of those plants which are necessary for the development of the particular grasshopper species. The habitat then may be changed in such a way as to lower its threshold of security, thus providing a less secure habitat or one which will support fewer individuals in secure surroundings.

It is realized, of course, that a great deal of information will have to be gathered before the conditions which produce an optimum habitat can be defined. What constitutes a secure or an insecure habitat can be judged only by the places a grasshopper selects when several choices are available. A knowledge of the behavior patterns of the parasites and predators should supply some of the missing information on this subject. At present we have some leads as to the general nature of security but its exact specifications, for even a single species, must await further research. It is interesting to note, however, that in every case Montana rangeland which is well managed from the standpoint of grass production does not develop a grasshopper problem, while lands which are abused provide at least some of the requirements for population build-ups. Observations reported from other areas indicate that the reverse may sometimes by true. Odum (1954) states that "rodents and grasshoppers increase with overgrazing. . . . Attempts to rehabilitate the range by rodent or insect control alone is a good example of what Leopold would call 'isolated project management,' which ignores the primary cause. Only by correcting the original excessive grazing pressure and considering the grassland community as a whole can permanent results be obtained."

Near the beginning of this paper it was pointed out that the discussion would be confined to a comparison between the behavior of higher animals and that of rangeland grasshoppers rather than attempting to include other insect species, many of which might have served as better examples. While many of the hypotheses which are presented are based on principles derived from studies on other animals, it is reasonable to do so because of the high degree of similarity between the basic behavior patterns in these widely separated classes of animals. With certain modifications it is possible that these principles may have quite wide application in the study of many insect species.

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Respiratory Protective Devices for Agricultural Use[1]

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Our interest in respiratory protective devices began at a meeting of the Interdepartmental Committee on Pest Control in 1949, when it was found that no suitable device was available for protection against organic phosphorus insecticides that were then being used on an experimental basis. In cooperation with manufacturers, all the absorbing materials and filters then available were tested to determine the type of unit that would remove the toxic vapors of these compounds, as well as chlorinated hydrocarbons, from sprays, mists, and dusts.

Two types of these protective devices are in use—respirators and gas masks. Respirators contain cartridges which are available either with a combination of a filter and absorbing material or separate filter and absorbing units, depending on the use. Gas-masks are equipped with canisters generally made with both a filter and absorbing material.

Respirators are designed to provide protection from dusts, mists, and low vapor concentrations of insecticides during field use, but not when one is formulating or mixing insecticides in close or inadequately ventilated spaces, or when applying insecticides, including aerosols, in closed spaces such as greenhouses. Full-face gas masks equipped with tested canisters should be worn under these indoor conditions.

In applying dusts and sprays under tobacco shade cloth, where the use of a gas-mask canister is necessary, operators have objected to the use of a full-face mask. For this purpose the respirator facepiece has been adapted to use the regular gas-mask canister. Tight-fitting goggles should be worn with this half-mask unit.

Testing Methods.—The apparatus for testing respirator devices consisted of a dusting or spraying chamber and a holder for the cartridge or gas-mask canister in series with a calibrated flowmeter. To determine the air resistance of the cartridges, a manometer filled with water was placed in the system after the cartridge. The air then passed through a sealed glass jar provided with an inlet and outlet which served as the test chamber for the insects. A vacuum pump maintained the air flow through the entire unit.

Bioassays were used in the tests with TEPP and parathion. Leaves infested with aphids were placed in small glass bottles filled with water. A 6-inch petri dish containing a piece of white paper was placed on the bottom of the glass jar. The bottles containing the infested leaves were set on this dish so that the fallen insects could be seen more readily. After the exposure period each leaf bearing the test insects was transferred to a vial standing in a beaker. A layer of paraffin supported the vial and provided a light-colored surface on which fallen insects were readily observed, and a coating of lanolin on the rim prevented the survivors from escaping. Mortality counts were made after 4 to 6 hours. Affected aphids were shriveled, and a yellow exudate was usually present at the opening of the cornicles. Mites were usually tested at the same time, but as the leaves were artificially infested mortality counts were always made within 30 hours after the mites had been transferred to the test leaves, to reduce the occurence of newly hatched nymphs or new adults.

Table 1.—Comparison of two types of filters in respirator cartridges against TEPP spray, as shown by bioassays.

Filter	Exposure Time	Aphids		Mites			
				Adults		Larvae	
		Dead	Alive	Dead	Alive	Dead	Alive
	Minutes	*Number*		*Number*		*Number*	
Fine-dust	60	322	0	43	2	320	0
Fume	60	3	129	1	186	0	243
	120	4	119	0	44	0	186

In the tests with parathion and chlorinated hydrocarbons the amount of material passing through the cartridge was determined by chemical methods. The parathion was determined by bubbling measured volumes (1 to 2 liters) of the test air through 5 ml. of absolute alcohol and comparing the optical density with known standards of parathion at a wave length of 274 millimicrons. The chlorinated hydrocarbons were analyzed by passing measured samples of the air through a quartz tube containing platinum foil heated to a bright red. The gases were absorbed in a tower containing glass beads wet with a chlorine-free solution of sodium carbonate containing arsenic trioxide (1 gram to 80 ml. of saturated sodium carbonate solution and 20 ml. of distilled water). Washings from the gas beads were transferred to a Nessler tube, acidified with nitric acid, and treated with an excess of silver nitrate solution, and the chlorine was determined by the turbidimetric method.

Tests with TEPP.—In tests with TEPP a 1:200 spray was applied at 5-minute intervals in the spray chamber, in which were green peach aphids on cabbage and two-spotted spider mites on beans. It was found that the fine-pore filters developed for the removal of metal fumes, when placed in front of activated-charcoal absorbing units, were more effective than absorbing units with large-pore built-in fine-dust filters (table 1).

Tests with Parathion Dusts.—Typical results obtained with parathion dusts are presented in table 2. Three types of cartridges were exposed to the dust-laden air in conjunction with the filters with which they would be used under practical conditions. The air was drawn

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Table 2.—Results of tests of respirator cartridges against 15% parathion dust.

Cartridge	Weight of Dust on Filter	Resistance to Air Flow		Parathion Passing Through Cartridge		Mortality of Aphids	
		Initial	Final	First 10 Minutes	Last 10 Minutes	First 15 Minutes	Last 15 Minutes
	Milligrams	*Inches of Water*		*Micrograms per Liter*		*Per Cent*	
A	172	0.5	1.0	0.45	0.45	61	18
B	185	.4	.75	.50	.50	35	22
C	180	.5	.81	.35	.40	42	28

Table 3.—Results of chemical tests of respirator cartridges with fine-dust filters and of a fume filter alone against three chlorinated hydrocarbon insecticides.[a]

Cartridge	Dieldrin		Aldrin Dust	Chlordane Dust
	Dust	Spray		
A	0.4	0.6	0.4	0.3
	.6	.4	—	—
	.2	—	—	—
B	.4	.8	.8	.3
C	.2	—	—	.3
	.3	.6	.3	.3
D	1.1[b]	—	—	—
Fume filter only	2.9	3.6	—	—
	1.2	—	—	—

[a] Micrograms per liter.
[b] 3 replications.

through the apparatus at the rate of 16 liters per minute, and the exposure period was 1 hour.

During the exposure period approximately 180 mg. of dust was collected on the filters. The resistance to air flow increased during these tests to a point where breathing would become difficult. The amount of parathion passing through the filter was considered to be within safe limits.

The decrease in aphid mortality from the first 15-minute exposure to the final one indicates that the filter became more efficient as the dust reduced the pore size, although no difference was detected by the chemical method. Exposures made without a cartridge killed all the aphids within 5 minutes.

When the fine-pore fume filters were tried alone against air saturated with parathion, they reduced the toxicant to less than 1 p.p.m., but an odor from the impurities was detected in the filtered air. Use of the activated-charcoal absorbing units removed the objectionable odor.

Tests with Chlorinated Hydrocarbons.—In tests with chlorinated hydrocarbon insecticides, dieldrin was used both in a dust and in a spray, but aldrin and chlordane only in dusts. The concentration was 5 per cent in all cases. The results of typical tests with several respirator cartridges in conjunction with fine-dust filters are shown in table 3.

Activated charcoal is very efficient in removing organic compounds containing chlorine. If the cartridge is designed to given uniform passage of air, as A, B, and C are, the units will have a long life. Unit D was not properly made, and the manufacturer has now modified the design to improve the efficiency. Fume-type filters alone had little effect on removing chlorinated materials.

Tests with Demeton.—Respirator cartridges and gas-mask canisters that had been recommended for use with the chlorinated hydrocarbons and other phosphorus compounds were found to be unsatisfactory for demeton. Three new types of filters were made available for testing and proved to be more effective than the fume type. These new filters are (1) glass wool impregnated with a special resin, (2) special fine-fiber wood pulp, and (3) a special grade of fine-fiber asbestos. The results of biological tests with these new filters against a spray of demeton in comparison with the fine-dust filter are shown in table 4.

Table 4.—Bioassay tests with new-type filters for protection against demeton spray.[a]

Filter	Aphids		Mites			
			Adults		Larvae	
	Dead	Alive	Dead	Alive	Dead	Alive
	Number		*Number*		*Number*	
Wood pulp	2	114	13	135	5	99
Impregnated glass wool	0	232	15	96	6	99
Asbestos	0	163	13	128	3	89
Fine-dust	196	3	98	7	78	2
Untreated check	0	153	13	128	3	89

[a] Exposure period 60 minutes.

Respirator and Gas-Mask Canisters Found Satisfactory

The following commercially available respirators have been tested and found to give adequate protection against dusts and mists and low vapor concentrations of aldrin, dieldrin, endrin, chlordane, EPN, parathion, and nicotine:

Respirator No. 5055 equipped with R-55 filter and cartridge unit. Two units attached to facepiece. American Optical Co., Southbridge, Mass.

Healthguard Respirator style 95 equipped with Code B cartridge and filter 1000 or 1001. One unit attached to facepiece. Chicago Eye Shield Co., 2300 Warren Blvd, Chicago, Ill.

Farm Spray Respirator equipped with filter and cartridge unit.[2] Two units attached to facepiece. Mine Safety Appliances Co., Braddock, Thomas and Meade Sts., Pittsburgh, Pa.

DC 5100 Aluminum Body Respirator equipped with DMA cartridge and P-7 filter. Two units attached to facepiece. Pulmosan Supply Equipment Corp., 644 Pacific St., Brooklyn 17, N. Y.

Agrisol Dust and Vapor Respirator equipped with R-414 filter and 11-A Agrisol cartridge. Two units attached to facepiece. Willson Products, Inc., Reading, Pa.

The respirators listed above do not give adequate protection from TEPP, or demeton vapors. However, the following units will give adequate protection against these materials as well as parathion, aldrin, dieldrin, endrin, chlordane, EPN, and nicotine. All of them have two cartridge units attached to the facepiece.

No. 5561 equipped with filter cartridge combination R-561. American Optical Co., Southbridge, Mass.

Farm Spray Respirator No. CR-72183 equipped with cartridge No. CR-49293 and filter No. 73488. Mine Safety Appliances Co., Pittsburgh, Pa.

All Vision Chemical Cartridge Respirator No. CR-74910 equipped with inner cartridge No. CR-73841 and outer cartridge No. 73927. Mine Safety Appliances Co., Pittsburgh, Pa.

Agritox Respirator equipped with cartridge No. 11A and filter No. R490. Willson Products, Inc., Reading, Pa.

The following gas-mask canisters have been found to give adequate protection against all the insecticides

[2] This respirator was unnumbered and equipped with a less efficient filter than No. CR-72183, which is listed for use against TEPP and demeton.

mentioned above under conditions where respirator cartridges would be inadequate.

Canister Type 2-OVAG or No. 15-OVAG-F Insecticide canister. Acme Protection Equipment Co., 3037 W. Lake St., Chicago, Ill.

Canister GMC-1. Mine Safety Appliances Co., Pittsburgh, Pa.

Canister G3FD. Willson Products, Inc., Reading, Pa.

Universal Type canister of any manufacturer. Type N, bearing Bureau of Mines approval.

Atomic Radiation for Insect Control

The recent experiment with screw-worms on the island of Curacao gives promise that atomic energy may provide entomologists with a new weapon for use in their continuous war against insects. The demonstration that this pest can be eradicated by the release of male flies made sexually sterile by irradiation will no doubt be the basis for further studies of the possibilities of this new approach to insect control—at least for a limited number of species. Radioisotopes have been used in various entomological studies, such as tagging insects in ecological experiments and physiological tests with radioactive insecticides, but this is their first use for insect control. Because this screw-worm experiment is regarded as an important milestone in entomological research, the three following papers, by the persons who have been responsible for different aspects of this study, are presented here.—EDITOR'S NOTE.

Possibilities of Insect Control or Eradication Through the Use of Sexually Sterile Males[1]

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The purpose of this paper is to consider the possibility of controlling insects by releasing sexually sterile males among the existing natural population. The principles involved will be described and the potentialities as well as the limitations of the method as we know them at present, will be discussed.

The theoretical possibilities of insect control by utilizing induced sterility or other damaging effects to genetic material have been considered by the writer for a number of years. Until recently there was insufficient experimental evidence to justify applied research to explore the method for pest control or elimination. However, the recent paper by Baumhover and coworkers (1955) presented at the 1954 meeting of the Entomological Society of America reported marked progress in the cooperative experiment to eradicate the screw-worm, *Calitroga hominivorax* (Cqrl.), from the 170-square-mile island of Curacao, Netherlands Antiles. A. W. Lindquist (1955) of the Entomology Research Branch, U. S. Department of Agriculture, announced the complete elimination of the insect on Curacao in a paper presented at the 1955 meeting of the Cotton States Branch, Entomological Society of America.

The experiment on Curacao was undertaken following 3 years of well-executed basic research under the leadership of R. C. Bushland of the Kerrville, Texas, laboratory of the Entomology Research Branch. Excellent cooperation was obtained from the Oak Ridge National Laboratory and the Netherlands Antilles Government.

Bushland & Hopkins (1951, 1953) published results of laboratory investigations which indicated the feasibility of studies such as those conducted on Curacao.

The studies by Bushland employing X-rays or gamma rays, confirming earlier investigations by geneticists and cytologists demonstrated that screw-worm males (and females) could be made sexually sterile by exposing pupae to irradiation without serious adverse effects on the mating behavior of the insect. The research also showed that the female screw-worm fly normally mates once only during her lifetime, and if mated to a sexually sterile male her reproductive potential is completely destroyed. It was demonstrated, for example, that when sterile and fertile males in a ratio of 4 to 1 were introduced in a cage of virgin females about 80 per cent of the females deposited eggs that did not hatch. The investigation by Bushland also showed that the sterile males produced sperms that fertilized the eggs but the developing larvae did not hatch.

After these key questions were answered, it was necessary to devise special research procedures and to carry out difficult and complex investigations in the field to determine if the principles established in the laboratory could be confirmed under field conditions. Early field work in Florida (unpublished) was encouraging, but the Curacao experiments by Baumhover and coworkers fully established the soundness of these principles.

The success of the Curacao investigations has created great interest, and inquiries are being received as to possibilities of this method for the control or eradication of other insects. It is hoped that information in this paper will stimulate further research where indicated and at the same time discourage investigations where the sterility method obviously will not be feasible. The writer is of the opinion that this method will be difficult and costly under the most favorable circumstances, but it might prove practical primarily as an eradication tool for certain highly destructive pests or provide a method of preventing the buildup or spread of established infestations. Basic studies on the problem might also, in future years, lead to new and more economical ways to induce sterility and thereby extend the practicability of this approach to insect control.

HOW STERILE MALES CAUSE A POPULATION DECLINE.

[1] Accepted for publication April 3, 1955.

—Before discussing the various requirements in estimating the feasibility of the sterilization technique now known, it seems important to describe the way insect control is achieved through the release of sterile males. The principle of reducing the population of an insect by the sustained release of sterile males among the existing population is a simple mathemetical proposition. The effect can be expected to be rapid and dramatic if circumstances are favorable and if the results follow theoretical possibilities. To illustrate this, some hypothetical figures are presented. It might be stated that calculations of this kind formed the basis for further investigations on the screw-worm. The figures presented below show the theoretical population trend of an insect in an area if sterile males are distributed in sufficient numbers to dominate the natural fertile male population initially by a ratio of 2 to 1, and if the number of sterile males released is maintained at a constant level as the natural population decreases. It is assumed in this example that the sex ratio in the wild population is approximately equal and that the released sterile males compete fully with wild fertile males in mating with the existing virgin females. It is also assumed that the wild population is essentially stable and that maximum depression of the population in subsequent generations will result.

Assumed Natural Population of Virgin Females in the Area	Number of Sterile Males Released Each Generation	Ratio of Sterile to Fertile Males Competing for Each Virgin Female	Percentage of Females Mated to Sterile Males	Theoretical Population of Fertile Females Each Subsequent Generation
1,000,000	2,000,000	2:1	66.7	333,333
333,333	2,000,000	6:1	85.7	47,619
47,619	2,000,000	42:1	97.7	1,107
1,107	2,000,000	1,807:1	99.95	Less than 1

It is apparent from the figures given that the sustained release of fully competing sterile males in a stable insect population could have a marked and rapid depressing effect. It is realized that a completely stable population is seldom encountered. However, many economic insect species have developed fairly close equilibrium with their environment. The full biotic potential of an insect is seldom reached, because of predation or parasitism, diseases, use of chemical control measures, cultural practices, limited host materials, and other factors. During periods favorable for a marked increase in population of a species, a ratio of 2 sterile to 1 fertile male might be insufficient to cause a depression in population of the succeeding generation, even though such releases might substantially reduce the rate of increase. For this reason a higher ratio would probably be indicated in most cases. In my opinion 9 sterile to 1 fertile insect should be released if possible to provide reasonable assurance of a downward trend in population of most insects. However, the optimum ratio will no doubt vary with the species, and the extent of normal population increase from one generation to the next. If initial release is properly timed to coincide with a natural or induced downward population trend, a low ratio of sterile to fertile insects might depress a population near the theoretical maximum. Subsequent releases at the same rate for one or two generations might then result in a sufficiently high ratio of sterile to fertile insects to continue the downward population trend even though conditions by that time, in the absence of sterile insects, would be favorable for a substantial increase. In order to determine the potential value of released sterile insects in control or eradication programs, population densities and population trends under various circumstances must be considered.

In the screw-worm eradication experiment on the island of Curacao the results were surprisingly close to maximum theoretical population depression. Within about 3 months after the eradication attempt was started, or in about three generations, the screw-worm was eliminated. The exact ratio of released sterile males to wild fertile males at the start of the experiment is not known, but it probably ranged between 2 and 4 to 1.

FACTORS TO CONSIDER IN APPRAISING FEASIBILITY OF INSECT CONTROL BY RELEASING STERILE MALES.—On the basis of the principle presented above we might list the factors to consider in appraising the possibilities of the method for controlling or eradicating an insect under various conditions.

1. An economical method of rearing millions of insects must be known or capable of development.

2. The insect must be of a type that can be readily dispersed so that released sterile males will be about as accessible to the virgin females in nature as are the competing fertile males.

3. The irradiation or other sterilization methods must produce sterility without serious adverse effects on the mating behavior or length of life of the males.

4. Females must normally mate only once. If females of a species mate more frequently, the sperms from irradiated (sterile) males must be produced in essentially the same number and compete with sperms from fertile males.

5. The insect to be controlled must have a low inherent population, or the species must under natural or induced circumstances reach a population sufficiently low to make it economically feasible to rear and release enough sterile males to effect a further downward trend in the population of subsequent generations.

In estimating the possibilities of releasing sterile insects of any given species, one must find the first four conditions favorable before considering the fifth. Failure to meet any one of the first four requirements will establish the impracticability of this procedure for insect control. However, if the first four requirements can be met, the practicability of the method will depend on the population density and the number of sterile insects that will be required to effect a downward trend in the population. The possibility of releasing a dominating sterile population might be considered for three types of circumstances.

1. *For controlling important pests that are normally present in small numbers.*—The screw-worm and the Australian sheep blow fly are examples of species where it might be economically feasible to rear and release sufficient sterile males to effect a downward trend in the population. Cattle grubs, *Hypoderma* spp., and other bot flies are present in relatively low numbers, but rearing methods may never be developed. It is possible that hornworm moths and tsetse flies in certain areas are present in sufficiently low numbers provided mass rearing methods can be developed.

2. *For newly established insect infestations before the infested area becomes large or the population density becomes high.*—The Mexican fruit fly infestation now present along the California-Mexico border is a type of incipient infestation where the method might conceivably be economical and effective.

3. *As an adjunct to other control methods.*—Several possibilities might be considered under such circumstances. In a small but intensive crop-production area special control efforts through the use of insecticides, biological control agents, or cultural practices might be employed to reduce an insect population to a low point, thus making it practical to rear and release a preponderance of sterile males.

The possibility of such procedure might be considered for various insects in which the cost of eradication would be justified. For example, in connection with the attempted eradication of *Anopheles* mosquitoes on Sardinia, a program sponsored by the Rockefeller Foundation, the use of insecticides reduced the population density of certain species to a point where it was difficult to find specimens, but complete elimination was not achieved. The regular distribution of a large number of sterile males in the area for several generations after the population had been greatly depressed might have destroyed the reproductive potential of the few females that escaped destruction by the insecticides.

Undoubtedly the intensive use of insecticides against such pests as the codling moth in areas such as the Yakima Valley in Washington reduces the population to a low density. If economical mass rearing methods at a cost comparable to that of the screw-worm or certain fruit flies were available, and if other circumstances should prove favorable, it might be practical to release sufficient sterile males in the spring to prevent the normal seasonal buildup. Releases for two or more seasons might eliminate infestations or reduce them to a point where routine releases to prevent economic population levels could be made more economically than spray schedules. Chances of success of such a procedure might be improved if a strain highly resistant to an insecticide could be reared, sterilized, and released in conjunction with regular spray schedules in the early stages of such program.

DISCUSSION.—It has not been demonstrated that the eradication of the screwworm will be economically feasible in any of the infested areas in the United States. We know, however, that the principle is sound so far as achieving eradication at a cost that approaches practicability. Further research on rearing techniques and release methods will no doubt reduce the unit-area cost below that in the Curacao experiment. It therefore seems worthwhile for investigators to give some thought to this method of controlling other pests.

The possibilities discussed in this paper are admittedly highly speculative. As pointed out by Bushland (1951, 1953), considerable information has been obtained by various investigators on the effects of ionizing irradiations on insects. Other workers, including Hassett & Jenkins (1952), Plough (1952), and Sullivan & Grosch (1953), have added to our knowledge of the subject. However, we know very little about the mating habits of most insects or the effects of gamma radiations on the activity of sperms. We have very little information on the variations in population density in terms of actual numbers in a given area or on the factors governing their biotic potential. Factors other than those mentioned must be considered. It probably would be impractical to release insects which are highly destructive in the adult stage. For insects having long life cycles the procedure would probably be too slow for practical consideration. It would not be realistic to enter into a costly eradication program unless practical ways of preventing reestablishment could be put into effect or unless it would be economically feasible to continue indefinitely releases in sufficient numbers of prevent a buildup of damaging numbers.

The problem of segregating sexes of an insect to avoid damage by the females or to increase the effectiveness of the released males has not been mentioned. This matter has been given considerable thought in connection with the screw-worm research project. Bushland & Hopkins (1951) showed that females of the screw-worm can also be made completely sterile. They also have shown that the presence of sterile (irradiated) females in caged populations of fertile and sterile males and unmated fertile females does not significantly reduce the number of unirradiated females producing sterile egg masses. It may be highly significant also that the gamma-irradiated females are not capable to depositing eggs, although they may attempt to oviposit.

Mass rearing and release of parasites as a means of controlling destructive pests is currently being given more consideration by entomologists. The release of parasites under conditions where there is an abundance of host material may result in a rapid increase in parasites of the following generation, thus increasing the ratio of parasites to hosts. This is no doubt an advantage over the male-sterilization procedure for reducing the population of certain insects. When the host insects are greatly reduced, parasites become less efficient. In some respects a sterile male of the species to be controlled can be regarded in the same light as a parasite. If a marked downward trend in the population is achieved by releasing sterile insects, and if such releases are maintained at a constant level, the ratio of sterile to fertile insects increases rapidly. Perhaps the greatest advantage of the sterilization technique over mass liberation of parasites is the probability that sterile males, because of the mating instinct, would seek out and destroy a few surviving females more effectively than the most efficient parasites known. The mass release of parasites or predators followed by the release of sterile males might be the most practical way to achieve control for certain pests.

Those who wish to consider employing sexually sterile

insects as a means of insect control by the procedures discussed should weigh carefully the many limiting factors before expending much research effort on such a problem. If the production and release of sterile insects might conceivably aid in control or eradication programs, exploratory research as a guide for further investigations would seem justified. Irradiation equipment, as described by Darden *et al.* (1954), which was employed in the screw-worm research may prove helpful in such preliminary investigations.

From a long-range viewpoint there is much that should be done to explore the feasibility of inducing sterility in insects in some manner among field populations. If practical ways could be developed by treating the natural population, thus obviating the necessity for rearing and distributing sterile insects, the feasibility of the method might be extended for a wide range of pests. For certain insects a chemical treatment to induce sterility instead of death would have great advantages over conventional insecticides. If, for example, a chemical spray or dust could be developed which would induce sterility instead of death in both sexes of the boll weevil when routinely applied to cotton, the treatment would not only destroy the biotic potential of the generation exposed but the infertile males would destroy the fertility of females which inevitably escape treatment. The addition to house fly baits of a chemical which would induce sterility would in time be more effective in fly control than a lethal toxicant. If a mobile irradiation unit could be developed which would cause sterility instead of death for all exposed pink bollworms in the cotton fields after harvest, such equipment would theoretically be far more effective in control or eradication programs than a similar device which would actually kill the insect.

SUMMARY.—Research on the screw-worm, *Callitroga hominivorax* (Cqrl.), has demonstrated that the release of large numbers of sexually sterile males in an insect population will reduce the existing natural population. The soundness of this principle as a potential means of controlling certain insects has been established. A number of key factors must be considered and resolved, however, before the procedure can be regarded as feasible for eradicating or controlling any given pest.

1. A method of mass rearing of the insect must be available.
2. Adequate dispersion of the released sterile males must be obtained.
3. The sterilization procedure must not adversely affect the mating behavior of the males.
4. The female of the insect to be controlled must normally mate only once, or if more frequent matings occur the sperms from gamma-irriadated males must compete with those from fertile males.
5. The population density of the insect must be inherently low or the population must be reduced by other means to a level which will make it economically feasible to release a dominant population of sterile males over an extended period of time.

Research to develop ways to induce sterility instead of death among field populations of pest species and the advantage of this approach over lethal measures is stressed.

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Screw-Worm Control Through Release of Sterilized Flies[1]

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Screw-worms, *Callitroga hominivorax* (Cqrl.), did not exist in the southeastern United States until about 20 years ago, and it is probable that, if the present infestation could be eradicated, the area might be kept free of infestation through inspection of livestock shipments originating in infested areas.

E. F. Knipling has suggested the possibility that screw-worms might be eradicated from the Southeastern States through the release of sterilized males. In average winters screw-worms survive only in peninsular Florida, and during the summer months infest areas to the north. Flies do not normally migrate from Texas to the Southeastern States.

Laboratory experiments (Bushland & Hopkins 1951, 1953) at Kerrville, Tex., showed that screw-worm flies could be sterilized by irradiation in the pupal stage with x-rays or gamma rays. Under cage conditions it was found that male screw-worm flies mated repeatedly but that

[1] This experiment was planned in conference with E. F. Knipling and A. W. Lindquist. The authors are further indebted to them for suggestions and guidance during the conduct of the work. Accepted for publication April 5, 1955.

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females mated only once. If a female mated with a sterilized male she laid only infertile eggs. When mixed populations of sterilized and normal insects were caged together, the ratio of fertile to infertile egg masses deposited by the normal females was about the same as the ratio of normal to sterile males in the cage.

These laboratory experiments were followed by field trials on Sanibel Island off the west coast of Florida. The results supported the laboratory findings. When sterilized males were released at the rate of 100 per square mile per week, the fertility of egg masses laid by the normal flies declined from the 100 per cent recorded prior to release to less than 25 per cent. After the eighth week of release the original fly population on the island appeared to have been eradicated. However, in the twelfth week a single fertile egg mass was collected.

Since Sanibel Island is only about 2 miles off the coast and is part of a chain of islands, it is within the flight range of insects from untreated areas. Therefore, efforts were made to find a properly isolated screw-worm population for a test to determine whether such a population could be eradicated through release of sterilized flies.

Officials of the Curacao Veterinary Service had sent specimens for identification by the Entomology Research Branch and requested advice concerning the screw-worm problem on the island. Since correspondence indicated that screw-worms were abundant there, the senior author made a trip to Curacao to conduct a survey. As a result of that visit the island administration and the Entomology Research Branch agreed to cooperate in this field experiment.

TEST AREA.—Curacao is an island of approximately 170 square miles about 40 miles off the coast of Venezuela. A constant trade wind blows out of the east. It was believed that the distance over water and the resistance of the wind were sufficient to prevent screw-worms from flying to Curacao from the mainland, and that if proper precautions were taken to prevent accidental importation of flies on cattle boats a valid experiment could be conducted.

Screw-worms were found to be extremely abundant on the island. They probably breed chiefly in wounds on neglected domestic animals. The island is estimated to have about 25,000 goats and 5,000 sheep, which roam through the brush and cactus largely unattended. Other potential hosts are a few hundred roving pigs, some neglected burros, about 300 deer, and numerous rabbits similar to our cottontails. There are also dairy cattle and horses on the island, but those animals, being fairly well cared for, are not considered important in breeding screw-worms.

The island is inhabited by about 115,000 people, most of whom work in town or for the oil refinery. Many of these workers live in the country and own small flocks of goats, which roam about in unfenced pastures. As there are no permanent streams on the island, during dry weather the goats must come to the houses for water except on those plantations having shallow wells in the pastures. Probably half the goats are seen daily when they come near the houses for water, but they do not receive much attention.

As the island is well served by roads, it was convenient to travel by automobile in conducting the field tests. There is an excellent airport. An airplane belonging to a flying club could be rented, and skilled pilots were available for distributing sterilized flies by air.

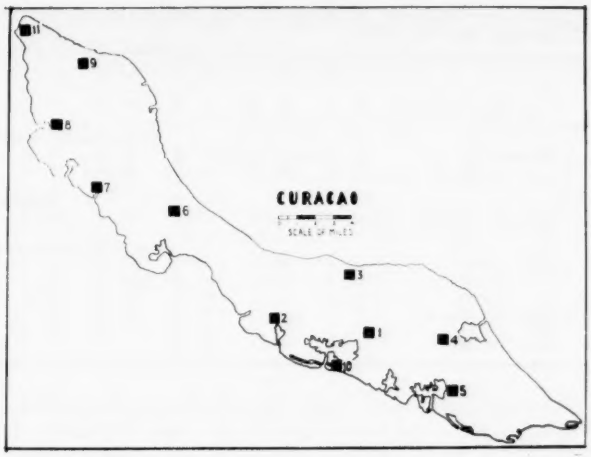

FIG. 1.—Map of Curacao showing location of goat pens.

ESTIMATION OF FLY ACTIVITY.—In this experiment procedures developed in the Florida work were followed. Experience had shown that fairly reliable estimates of fly activity could be made by exposing wounded goats to oviposition by flies in the natural population.

Goat pens were established in 11 locations distributed over the island, as shown in figure 1. Each pen consisted of a fenced enclosure about 20 feet square, in which eight goats were maintained. Each week two goats in each pen were wounded and artificially infested with approximately 100 newly hatched screw-worm larvae. When the larvae were 3 days old, the wounds were fumigated with benzol to destroy the larvae. The wounds were attractive to ovipositing flies from the time of infestation until several days after the benzol treatment.

At the beginning of the experiment in each goat pen there were always at least two fairly fresh wounds and two healing wounds to attract ovipositing flies. Later in the work infested goats were not kept in the pens to avoid the risk of escape. From that time goats were wounded and infested in a locked enclosure and only brought to the field after larvae had been removed from the wounds on the third day.

The goat pens were visited late in the afternoon each day for the purpose of collecting egg masses. Individual egg masses were removed and placed on moist filter paper and held in a petri dish for observations on hatching. Sometimes eggs were laid at a pen after the observer had made his visit. Early in the work, when fertile egg masses were common, such egg masses hatched and the shells usually fell off before the next inspection. The observer could recognize such missed egg masses by the presence of newly hatched larvae in the wound. Infertile egg masses, which did not hatch, remained intact.

Early in the experiment there were undoubtedly errors in estimating egg masses. If eggs hatched prior to a visit, one could only estimate the number of egg masses on the basis of the number of larvae he found in the wound.

Table 1.—Numbers of fertile (F) and sterile (S) screw-worm egg masses collected in goat pens on Curacao from March 17 through May 18, 1954.

Date	No. 1		No. 2		No. 3		No. 4		No. 5		No. 6		No. 7		No. 8		No. 9		No. 10		Totals		Per Cent Sterile
	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	
Oviposition before release of sterilized males																							
March 17–23	12	0	37	0	8	0	6	0	13	0	11	0	8	0	14	0	15	0	9	0	133	0	0
24–30	9	0	32	0	8	0	8	0	18	0	20	0	15	0	21	0	16	0	8	0	155	0	0
Transition period																							
March 31–April 6	11	0	48	1	7	1	2	0	13	2	20	0	7	0	19	0	6	0	1	0	134	4	3.0
Oviposition following release of sterilized males																							
April 7–13	5	2	26	2	9	4	12	4	12	1	14	3	4	0	10	0	4	0	8	1	104	17	14.0
14–20	18	6	24	8	30	8	11	2	9	4	25	4	14	4	16	5	22	4	14	1	183	46	20.1
21–27	22	2	8	1	35	5	6	0	6	1	24	1	21	4	30	7	28	4	5	3	185	28	13.1
April 28–May 4	27	1	11	2	23	3	24	3	13	1	15	0	4	1	23	6	14	2	8	4	162	23	12.4
May 5–11	26	6	20	4	39	7	6	3	11	2	24	0	8	4	26	1	33	3	7	9	200	39	16.3
12–18	41	7	29	5	23	8	9	1	11	3	19	1	34	2	40	7	30	3	4	0	240	37	14.4

When egg masses were thickly deposited around a wound, the observer could not always count them accurately if he did not know whether a given clump of eggs represented one large or two small masses. However, it is believed that those sources of error were minor.

REARING AND STERILIZATION OF FLIES.—Insects for release were reared and sterilized at the Orlando, Florida, laboratory. The flies used were of a strain originally started at Kerrville, Texas.

The larvae were reared on a mixture of ground horse meat, blood, and water by a modification of the procedure described by Melvin & Bushland (1941). The pupae were held at a constant temperature of 80° F., and when the pupae were 5 days old they were sterilized with gamma rays, using the cobalt-60 irradiation unit described by Darden *et al.* (1954). During the experiment three different doses of sterilizing radiation were employed—5,000 6,000 and 7,500 r. Although flies sterilized with 5,000 r could not produce normal egg masses, they sometimes laid a very few eggs, which caused confusion in record taking when the normal population was near destruction. When the sterilizing dose was increased to 7,500 r, females incapable of ovipositing more than one egg were produced.

Immediately after irradiation the pupae were packed with a little excelsior in No. 1 kraft paper sacks, 130 insects in each sack, and shipped by air freight to Curacao, where they emerged in the sacks and expanded their wings on the excelsior provided.

Within a few hours after emergence was completed, the flies were distributed by airplane. The pilot flew at a height of about 400 feet in flight lanes 1 mile apart. An entomologist riding in the airplane tore open the sacks and dropped them through a pipe which extended below the fuselage. The bottoms of the sacks were smeared with a paste of sand and flour to weight them so that they would drop without drifting unduly. This method of dispersal seemed quite satisfactory, as flies dropped in intact sacks appeared unharmed by the fall.

In the first phases of the experiment the flies were not fed prior to release, but later it was thought desirable to provide the insects with food and water. This was done by packing the insects in sacks with wire-screen bottoms through which they could be fed.

Flies were distributed twice each week. On alternate flights the flight lanes were shifted so that the flies were distributed in lanes ½ mile apart each week.

The packaging rate of 130 pupae per sack was selected on the expectation that out of that number about 100 flies would emerge. Thus, when 50 males and 50 females were expected in each sack, sacks dropped at 1-mile intervals represented a distribution of 50 males per square mile. The actual percentage emergence was determined on sacks retained in the laboratory, and the exact rate of release was calculated subsequently. Thus the rate of fly distribution was regulated by the number of sacks dropped per mile in flight lanes 1 mile apart.

EFFECTS OF RELEASING 100 STERILIZED MALES PER SQUARE MILE PER WEEK.—By March 17 ten goat pens were established over the island, and during the following week 133 fertile egg masses were recorded. Just what adult fly density such a figure represents is not known, but that rate of egg-mass deposition was seldom equaled in any experiment in Florida. Beginning on March 26, flies sterilized with 7,500 r of gamma rays were distributed. During the second week 155 egg masses, all of them fertile, were collected. The week of March 31 to April 6 represented a transition period, since some of the ovipositing flies might be young females that had mated since release of sterilized flies began, whereas others would be older insects that had already mated. During that third week 138 egg masses were collected, and of these 4 were sterile. Semiweekly releases of sterilized flies at the rate of 100 per square mile per week were continued until May 7.

The records on egg masses are summarized in table 1. The data are remarkably uniform. They definitely show that some of the released males mated with females of the native population, but since egg-mass collections increased rather than declined it appeared that the proportion of sterile matings was not sufficient to cause a decline in the population.

Releasing sterilized males at the rate of approximately 100 (actually 99) per square mile per week along with an equal number of sterile females caused about 15 per cent of the native flies to lay egg masses that failed to hatch. It should not be concluded that those sterile males represented only 15 per cent of the male population. Undoubtedly many males died soon after distribution before they could find water and food. In observations in the United States normal males lived longer in the laboratory than did sterilized males, and this was probably true in Curacao. Therefore, it should not be assumed that, because only about one-sixth of the Curacao females laid

Table 2.—A comparison of releasing sterilized male screw-worm flies at rates of about 100 and 400 per square mile per week.

Date	100 Males per Week		400 Males per Week	
	Average Number of Egg Masses per Pen	Per Cent Sterile	Average Number of Egg Masses per Pen	Per Cent Sterile
July 19–25	10.5	30.2	16.0	37.5
July 26–Aug. 1	9.5	33.3	11.8	55.9
Aug. 2–8	10.0	28.3	6.0	53.3
Average	10.0	30.6	11.3	48.9

infertile eggs during the period of release at the rate of 100 males per square mile per week, there were about 500 normal males per square mile in the normal population. Probably the normal population was much smaller but merely lived longer and competed more effectively for mates than the released flies.

EFFECT OF WEATHER ON FLY ACTIVITY.—From the middle of May until July 12 there were no regular releases of sterilized flies on the island. During the first month some special tests were performed which indicated that males sterilized with 7,500 r mated as efficiently as those treated with 5,000 r. During the next 4 weeks no flies were released. Also during this time the eleventh goat pen was established so that the northwest end of the island could be adequately sampled for egg-mass data. From mid-May to mid-July the egg-mass collections indicated a natural decline in the fly population, as collections dropped from 188 to 70 per week.

The decline in the fly population is attributed to the dry weather. After the first week in May there was almost no rainfall until the middle of June. After June 14 rains were frequent, and for the remainder of the year the weather was very favorable for screw-worms, so that any further decline in the fly population could be attributed to sterile matings. In previous years screw-worms had been observed to increase from July to January.

COMPARISON OF 100 AND 400 RATES OF RELEASE.—For 4 weeks beginning July 12 a comparison was made between two rates of releasing males. In the southern half of the island the flies were released twice weekly at the rate of 100 per square mile per week and in the northern half at 400 per square mile per week. When emergence figures were calculated from final laboratory records, the actual releases were found to be 107 and 450 per square mile per week.

From the results of this experiment, shown in table 2, it will be noted that the lower rate was more effective than it was in the spring when the natural population was higher. In the first experiment (Table 1) releasing at the rate of approximately 100 per square mile caused 22 per cent sterility among egg masses collected on the southern half of the island. In the second experiment the sterility from a similar release rate was 31 per cent, and from the 400 release rate it was 49 per cent.

The record of 49 per cent sterility of egg masses collected on the northern half of the island indicated that such a reduction in breeding potential should cause a decline in the fly population.

Table 3.—Egg mass records during release of sterilized males over island of Curacao.

Date	Males Released per Square Mile	Total Egg Masses in 11 Goat Pens		
		Fertile	Sterile	% Sterile
Aug. 9–15	491	15	34	69
16–22	224	17	38	69
23–29	175	17	36	68
Aug. 30–Sept. 5	381	10	37	79
Sept. 6–12	451	7	42	86
13–19	701	3	23	88
20–26	450	0	10	100
Sept. 27–Oct. 3	607	0	12	100
Oct. 4–10	484	0	0	—
11–17	727	0	0	—
18–24	577	0	0	—
Oct. 25–31	400	0	0	—
Nov. 1–7	400	0	1	100
8–14	485	0	1	100
15–21	393	0	0	—
22–28	400	0	0	—
Nov. 29–Dec. 5	421	0	0	—
Dec. 6–12	498	0	0	—
13–19	313	0	0	—
20–26	265	0	0	—
Dec. 27–Jan. 2	400	0	0	—
Jan. 3–6	188	0	0	—

ERADICATION EXPERIMENT.—Beginning on August 9 sterile flies were released over the entire island at the approximate rate of 400 males plus 400 females per square mile per week. Attaining this release rate required the actual release of 68,000 of each sex over the entire island, or 136,000 flies per week. It was necessary to ship twice that many flies to compensate for losses due to conditions beyond our control. Occasionally the insects emerged en route, either because the shipments were delayed or because the packages were exposed to unusually high temperatures. Rearing, irradiating, and packing the insects at the rate of 130 pupae per release sack strained the capacity of the Orlando laboratory. Some weeks it was impossible to meet the release quota because of rearing or shipping mishaps. In order to attain the desired average it was necessary to release practically all the insects that reached Curacao in good condition. For that reason weekly releases varied greatly. During the first 8 weeks, in which screw-worms were practically eliminated from Curacao, the weekly releases ranged from 175 to 701 males per square mile and the average was 435. The figures for each week's release are shown in table 3.

From October 3 until November 4 no egg masses were collected, but a single infertile egg mass was taken at goat pen No. 6 on November 4 and another at goat pen No. 4 on November 11. After November 11 there was no oviposition on the island detectable by the sampling procedure.

Under weather conditions prevailing on Curacao, about the shortest time for a complete life cycle from egg to egg is 3 weeks, and 4 weeks is about the average. The egg-mass data show that fertile flies were active until mid-

September. As there are about 25,000 goats and 5,000 sheep roaming on the island, it would be expected that, if fertile females were ovipositing on observed wounds at the goat pens, many more should be ovipositing on unattended wounds in nature. Flies ovipositing on neglected sheep or goats or on wild mammals should have produced progeny to oviposit a month later in mid-October. The fact that no egg masses were collected after October 3 is a good indication that the native fly population was greatly reduced, but it also suggests that the 11 goat pens under observation did not provide an adequate sample of a declining fly population when the number of ovipositing females was very small.

The females that oviposited on November 4 and 11 probably were the progeny of flies ovipositing early in October. It is possible, but not likely, that the two egg masses were laid by flies 3 or 4 weeks old and hence from parents that oviposited in early September. Since the records were taken at widely separated pens, they probably represented different centers of survival.

Maintenance of the goat pens and systematic releases of sterilized flies were continued through January 6, 1955. Thus, observations were made for 8 weeks after the last record of oviposition. This was ample time for two complete life cycles.

It is quite probable that the two flies that oviposited on November 4 and 11 had brothers and sisters active at the same time. It seems, though, that there were no fertile eggs laid on animals in nature in November, as 6 months have elapsed (May 1955) without further evidence of normal flies.

In addition to taking the egg mass records at goat pens, livestock owners were questioned regarding the occurrence of screw-worm cases in their animals. Very few cases of myiasis were reported after October 1, and every one proved to be due to an infestation of *Callitroga macellaria* (F.) rather than *hominivorax*. This checking with livestock owners is being continued by one of us (B. A. Bitter), but to this writing (May 1955) there has been no evidence of renewed screw-worm activity.

During the course of this experiment there was an obvious increase in the number of surviving kid goats. At the beginning of the study less than half the nannies had kids and twins were seldom seen, but at the conclusion most of the nannies had kids and twins were common. Livestock owners remarked that it had been their practice to seek out new-born kids for treatment with larvicides to prevent almost certain death through navel infestation. As the screw-worm incidence declined the owners abandoned that practice and all remarked at the great increase in the kid crop.

CONCLUSION.—It appears that screw-worms were eradicated from the island of Curacao through the release of sterilized flies. The success of this experiment strongly supports the theory that it may be practical to eradicate screw-worms from the Southeastern States by this method.

SUMMARY.—To test whether an isolated population of screw-worms, *Callitroga hominivorax* (Cqrl.) might be eradicated through the release of sterilized flies, experiments were made on the island of Curacao.

Screw-worms were reared in the laboratory at Orlando, Florida. When the pupae were 5 days old they were sterilized with gamma rays and shipped by air freight to Curacao. The adults emerged 2 days after irradiation. Within 24 hours of emergence the insects were distributed by airplane over the island.

When sterilized flies were released at the rate of approximately 100 of each sex per square mile per week, some of the males mated with females of the normal population, as indicated by the deposition of infertile egg masses on wounded goats.

Egg masses collected from wounded goats prior to release of sterilized flies hatched 100 per cent.

During April and May, when weather favored fly activity, releasing sterilized males at the rate of about 100 per square mile per week caused 15 per cent of the females to lay infertile eggs on wounded goats in pens. This rate of sterility was not sufficient to cause a reduction in the fly population.

At the end of the dry season in July and August releasing at 107 per square mile per week over half the island caused 30.6 per cent sterility, and 450 per square mile per week on the other half caused 48.9 per cent sterility.

From August 9 to October 3 the entire island was treated at a rate that averaged 435 males per square mile per week. Although frequent rains made conditions suitable for fly increase, the releases were highly effective. In 8 weeks the percentage of sterile egg masses increased from 69 to 100 and the population declined more than 99 per cent. The releases were continued. Except for two sterile egg masses collected after 12 and 13 weeks, there was no evidence of normal fly activity after the eighth week of releases. The releases and observations were discontinued 8 weeks after the last egg mass was collected.

The apparent eradication of screw-worms from Curacao supports the theory that screw-worms may be eradicated from the Southeastern States by releasing sterilized flies.

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The Use of Gamma Radiation for Control or Eradication of the Screw-Worm[1]

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The screw-worm, *Callitroga hominivorax* (Cqrl.), has always been a serious pest of livestock in the Southwest, but it was not found in the Southeastern States until 1933. Undoubtedly it was introduced into this region by livestock shipments from the Southwest. Since its establishment in the Southeast, the screw-worm has caused serious losses to stockmen in Florida, Georgia, Alabama, and the Carolinas. Inasmuch as the species is not native to these states, it seems that if the present infestation could be eliminated they might be kept free from reinfestation through inspection of livestock shipments originating in or near infested areas.

The possibility of eradicating the screw-worm from the Southeast has been under consideration since its discovery there. Rigid livestock-management practices have been discussed, but they would be extremely costly and probably would not succeed because of infestations in wild host reservoirs, which cannot be controlled.

The purpose of this paper is to review the results of experimental work conducted by the former Bureau of Entomology and Plant Quarantine and its successor, the Entomology Research Branch, on the idea, first propounded by E. F. Knipling about 1938, that control and perhaps eradication of a natural insect population could be achieved through the release of sterilized males of the same species. These sterilized males would mate with normal females, but the eggs would be infertile and therefore not develop. The success of such a method obviously depends upon many factors, but there are three basic requirements: (1) A low natural population at some period of the year is essential; (2) females should preferably mate only once; (3) the insect must be easily reared in the laboratory. Dr. Knipling believed that the screw-worm might lend itself to a demonstration of the effectiveness of the method. Biological and other work conducted many years ago was then reviewed to assess the possibilities of control by means of sterilization.

REVIEW OF ECOLOGICAL WORK.—During the years 1932–38 personnel of the Uvalde, Tex., laboratory determined that the number of screw-worm flies per square mile probably was only a few hundred rather than thousands or hundreds of thousands as with many economic pests. Fairly accurate estimates on the incidence of screw-worm cases in various classes of livestock were available. Since the numbers of livestock present in an area were known, it was possible to obtain reliable data on the number of screw-worm cases per week and hence to estimate with a reasonable degree of accuracy the number of larvae produced.

Many data were obtained on screw-worm incidence in deer, rabbits, opossums, and other wild animals in Uvalde County (Lindquist 1937). These data indicated that from 50 to 90 per cent of the screw-worm flies probably came from wild animal hosts.

Another significant finding was that most of the screw-worm larvae dropping from wounds were destroyed by ants. Usually only 5 to 20 per cent of them survived and produced flies. This was further evidence that the fly was comparatively scarce in nature. A study of all the data on numbers of screw-worms in domestic and wild animals and on their survival suggested that in Uvalde County, which is known for its high screw-worm incidence, only 100 to 500 flies were produced per square mile per week during the height of the season.

Extensive surveys conducted as a part of the screw-worm control program in the Southeast during the mid-thirties also provided much information on the number of screw-worm cases reported in livestock during various seasons of the year. Excellent surveys were made by Bruce *et al.* (1947) and Smith & Skipper (1951).

LABORATORY STUDIES.—It was not until 1950 that laboratory experiments with X-rays were initiated to obtain the necessary basic information on sterilization. Geneticists had known for years that overdoses of X-rays caused extreme changes in genes and chromosomes of *Drosophila*.

Bushland & Hopkins (1951) reported that screw-worm pupae exposed to certain amounts of X-ray irradiation became sterile adult flies. They found the best time to irradiate pupae to be within 2 days of emergence. In laboratory cage tests the sterile males competed satisfactorily with the normal males in mating with normal females. When the ratio of sterile to fertile males was 5 or 10 to 1, the ratio of sterile to fertile egg masses was essentially the same. They also demonstrated that female screw-worm flies mate only once, whether with a sterile or a fertile male. These findings were important milestones, but it was obvious that the expense of sterilizing large numbers of screw-worm pupae with X-rays would be prohibitive. A search was therefore made for an inexpensive source of radiant energy.

The development of gamma-ray sources by the Atomic Energy Commission offered possibilities. Through the cooperation of the Biology Division of Oak Ridge National Laboratory a cobalt-60 source of gamma rays was obtained for experimentation. Bushland & Hopkins (1953) reported on the use of gamma rays from this source for sterilizing screw-worm pupae. The source of energy was found practical and reasonably inexpensive. A dose of 2,500 roentgens caused sterility of the males and 5,000 roentgens sterility of the females. The optimum time for exposure of pupae was determined as 5 days after pupation at 80° F., or 2 to 3 days before emergence of the adults. As far as could be determined, the sterilization did not seriously injure the adults but it apparently reduced their longevity. Cage tests showed that the gamma-irradiated males competed for mates about equally with the normal males, confirming the observations made with X-ray-treated males.

FIELD STUDIES.—During the winters of 1951–52 and 1952–53 personnel of the Kerrville, Tex., laboratory

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conducted field tests to explore the possibility of reducing the native screw-worm population by releasing sterilized males on two small adjacent islands, Sanibel and Captiva, about 3 miles off the lower west coast of Florida. Sterile flies were also released on some nearby islands and on the coast of the mainland opposite the two test islands, in order to reduce the possibility of migration into the main test areas. The release area totaled about 75 square miles. The flies were reared and sterilized by exposure to gamma rays from a cobalt-60 source at Orlando and sent by air to the test area, where they were distributed systematically from an airplane. Males only were released at the rate of 100 per square mile per week on the two test islands and 50 per square mile per week on the nearby islands and the mainland.

In 8 weeks 80 per cent of the egg masses deposited on wounded goats placed on Sanibel Island were sterile, and on Captiva Island 35 per cent sterility was obtained. The islands were not sufficiently isolated to prevent migration of flies onto or off the land area. These tests demonstrated that screw-worm flies could be greatly reduced in a short time, but several questions remained unanswered relative to the most efficient procedures and the possibility of eradication by continuous releases. It was necessary to experiment on a completely isolated island.

The island chosen was Curacao, a 170-square-mile possession of the Netherlands in the Caribbean Sea about 50 miles off the coast of Venezuela. This island is semiarid and has vegetation and topographical features similar to those of parts of southwestern Texas. Screw-worm flies were present throughout the year, and the incidence of cases in goats and cattle appeared to be similar to that in Texas.

Although much information on the sterilization procedure had been accumulated, numerous questions and problems confronted us. Organizing the program was one of them. R. C. Bushland, in charge of the Kerrville laboratory, was assigned the responsibility of directing the island experiment. The Netherlands Government cooperated generously in many ways, especially by furnishing personnel and equipment. B. A. Ritter, of the Netherlands Government, and A. H. Baumhover and Weston D. New, of the Kerrville laboratory, handled the insects upon receipt from Florida, released them systematically by airplane, and obtained daily oviposition records on dozens of experimental goats located over the island.

The rearing and sterilizing of the insects were done at the Orlando laboratory by A. J. Graham, D. E. Hopkins, and F. H. Dudley. They reared from 150 to 200 thousand screw-worms per week in an artificial medium, which was not only a time-consuming job but one requiring constant care and attention to numerous details.

The research program on the island was begun in April 1954 (Baumhover *et al.* 1955). The first efforts were directed toward estimating the natural fly population. The effect of releasing small numbers of sterilized flies on the native population was then determined. Because of the time and labor required to separate the sexes, both sexes were released. These preliminary releases reduced the population about as expected, and indicated that eradication could be achieved with higher release rates.

An all-out attempt at eradication was therefore started early in August. Sterilized males were released at the rate of about 400 per square mile per week until January 7, 1955. Shortly after these releases were started the number of egg masses laid on goats began to decrease and their sterility increased proportionally. By mid-September, or only 7 weeks after releases were started, all the eggs were infertile. The total number of egg masses, which averaged about 150 per week in July, decreased steadily to zero by October 4. Thus in 2 months the native fly population had been suppressed to a point where it was impossible to find an egg mass. Throughout October and November no screw-worm cases in livestock were found or reported by local people. It is highly significant that this decrease was accomplished during the rainy season, when screw-worm cases were usually increasing.

On November 4 and 11 two small infertile egg masses were obtained from trap goats. Therefore, it was decided to continue releases for an additional 8 weeks in an effort to assure eradication. No further egg masses were found, and on January 7, 1955, the release of flies was discontinued. At this time no fertile eggs had been collected since mid-October. Moreover, no screw-worm-infested animals were observed during this period. Since there has been no evidence of fertile screw-worm flies or infestations during a period sufficient for the development of about four generations of this insect, it is believed that the screw-worm has been eradicated from Curacao.

The period required to achieve apparent eradication by the release of sterilized flies was less than 5 months. Perhaps in no other way could eradication be achieved so surely as by exploiting the mating habits of these insects. This highly effective means of seeking out native females and destroying their reproducibility may not be applicable to many insects, but it is a new approach to control that will undoubtedly be explored by entomologists throughout the world.

POSSIBILITY OF USING METHOD IN FLORIDA.—The successful elimination of the screw-worm from Curacao raises the question whether the same method could be used on the Florida mainland. The cost of a similar program for the entire state of Florida would be considerable. In years with below-average temperatures, however, the screw-worm survives the winter only in the southern two-thirds of the State, and it is believed that eradication might be achieved under these conditions at less cost than the monetary loss to the livestock industry in the Southeast from this insect for a single year. The area to be covered in Florida would be about 50,000 square miles, or 300 times that of Curacao. The problem of expanding the program 300 times presents numerous obstacles, but we believe they can be overcome.

In course of the Curacao experiment we learned much and gained invaluable operational experience, but we are not yet ready to recommend a large-scale eradication program. Another year or two of research is needed to develop better methods for determining natural fly populations and improved and cheaper rearing media. A search should be made for a screw-worm attractant

to take the place of wounds on animals for detecting the presence and incidence of flies. A means of estimating the native population of flies is necessary to know the number of sterile flies to release in a given area. In addition, a large-scale experiment similar to the one on Curacao should be conducted in Florida. The test area should be about 2,000 square miles, the objectives being to test mass rearing and release procedures and to determine whether this principle would be suitable for an eradication program involving most of Florida.

An expanded research program of about 2 years' duration is therefore necessary before recommendations can be made as to the practicability of a screw-worm eradication attempt in the Southeast. At this time the obstacles do not appear insurmountable and, given time and funds, the second phase of these studies on utilizing sterile screw-worm males for elimination of a natural population should be successful. The future of this idea is very promising.

POSSIBILITY OF TEMPORARY CONTROL IN TEXAS.—Although the research thus far has been directed toward elimination of the species in selected or isolated areas, at some future time consideration may be given to use of the method as a temporary control in such places as Texas. Eradication in Texas would not be practical, since the insect would migrate from Mexico and reinfest the area. Control in overwintering areas in southwestern Texas might prevent spread to other areas. Obviously control efforts would have to be made over thousands of square miles.

In any control effort costs and ease of applying treatments are important considerations. At present only rough estimates of the costs of rearing, sterilizing, and releasing of screw-worms are available. The artificial medium in which screw-worms are now reared is expensive, since horsemeat or beef is the main ingredient. Research is needed to find cheaper materials. Rearing methods undoubtedly could be improved, perhaps to make the operation more or less automatic, thus reducing the manpower required. Costs in handling large numbers of flies before and during releases can no doubt be reduced. Research directed toward cost reduction and streamlining the various steps in large-scale production would probably pay for itself many times over.

At present, therefore, it is impossible to predict that the release of sterilized screw-worm flies for temporary control is feasible or practical. After further research has established the costs to be expected, the livestock industry should determine whether the results would justify such expenditures.

Wildlife conservationists and hunters, as well as the stockmen, would have an interest in screw-worm control on a large-area basis. In many counties of Texas screw-worms destroy from 5 to 20 per cent of the deer crop each year. Many ranchers realize a good income from selling hunting leases to sportsmen, and usually the more deer available the better are the prices on hunting leases. It is likely that screw-worm control on an area basis would increase the number of deer in many Texas counties. These groups might join with the livestock industry in encouraging and supporting a control program.

SUMMARY.—The possibility of eradicating the screw-worm, *Callitroga hominivorax* (Cqrl.), from the Southeastern States has been under consideration since it was introduced there in 1933. Since the screw-worm has a comparatively low natural population, it seemed that the release of more sterilized males than occurred in nature might result in eradication. Research conducted by the Entomology Research Branch showed that sterilized males released over small areas mated with native females and the eggs produced were infertile. In an experiment conducted on the island of Curacao the release of sterilized males at the rate of approximately 400 per square mile per week for 7 weeks caused an initial sterility of about 70 per cent which increased to 100 per cent sterility as the number of flies and egg masses declined. Releases were continued for 3 more months, during which time no eggs, flies, or screw-worm cases could be found. Apparently eradication was achieved during a time of the year when the infestation normally increases. The possibility of using this method of eradication in Florida is promising.

Eradication in Texas does not appear practical because the screw-worm is present in Mexico. Temporary control might be achieved in overwintering areas of Texas, but the practicability of such an effort is dependent on costs of rearing and releasing screw-worms.

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Co-ordinated Experiments on Chemical Control of Root Maggots in Rutabagas in British Columbia and Washington, 1953[1]

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King & Forbes (1954) reported on extensive experimental work carried out from 1948 to 1952 on Vancouver Island, B.C., in which they were able to control root maggots in rutabagas by applying aldrin or heptachlor by any one of several methods. In addition they clarified the problem of root maggot attack on rutabagas and developed methods for appraising the damage and control on this crop.

With that work as a basis, a common experimental design was drawn up and co-operative experiments to test the controls under varying climatic and local conditions were carried out in 1953 at five localities: Victoria, Courtenay, Agassiz, and Kelowna, B.C., and Puyallup, Washington. Each research officer was free to include additional treatments in the test. The chief species causing damage to rutabagas in all the experiments was *Hylemya brassicae* (Bouché) although *H. floralis* (Fall.) also occurred at Courtenay during August and September.

Materials and Methods.—The Laurentian variety of rutabagas (swede turnips) was used in all experiments.

Plots consisted of 50 feet of rutabaga row and a randomized block design was used throughout. There were five replications at Victoria, Courtenay, Agassiz, and Puyallup and four at Kelowna. Seeding was done with a Planet Junior seeder at all localities except Kelowna, where a rod-row multiple-gear seeder was used. Dates of seeding were as follows:—Victoria, June 23; Courtenay, June 16; Agassiz, June 17; Kelowna, June 25, 26; Puyallup, June 15.

Insecticides were applied in four ways. By the *band method* a single application of insecticide dust was raked to a depth of about 1 inch into a 10- or 12-inch band, and the seed was planted immediately, along the center of the treated area. By the *furrow method* insecticide dust was applied once, in the planting furrow with the seed: the planting furrow was opened with a hoe, the insecticide was evenly distributed in the furrow, and the row was immediately seeded and covered with the drill, except at Kelowna where the insecticide was placed in the V belt of a rod-row multiple-gear seeder and applied with the seed. The *spray method* was of two types. At Victoria, Courtenay, and Puyallup there were two applications each of 200 imperial gallons per acre, to the crowns of the plants and adjacent soil surface: the first application was made immediately after an early thinning, i.e., about 2 or 3 weeks after seeding, specifically July 13, 7, and 17; the second application was made when crop growth was well advanced but would still have permitted the practical use of power spraying equipment, specifically August 11, 14, and 10. At Kelowna there were seven applications, each of 100 gallons per acre, to the foliage at 10-day intervals commencing on July 11, when the plants were about 1 inch high. By the *seed treatment* method (Kelowna), the seed was first moistened with water and then mixed with a specified amount of chemical until all the chemical adhered to the seed.

The insecticide used, the method of application, and the rate for each treatment are given in table 1; the rates per acre as shown are based on 14,520 lineal feet of row per acre, the rows being 3 feet apart. All the insecticides were used in standard formulations.[5]

Table 1.—Insecticides against root maggots injuring rutabagas in five localities, British Columbia and Washington, 1953.

Method and Chemical	Toxicant[a] per Acre (Pounds)	Mean Damage Index[b]					Average Control (Per Cent)
		Victoria	Courtenay	Agassiz	Kelowna	Puyallup	
Band							
Aldrin	.5	24	9	9	7	1	89
Heptachlor	.5	21	7	2	4	0	92
Heptachlor granulated	.5	86	71	74	—	—	21
Furrow							
Aldrin	2.5	31	6	11	22	4	83
Heptachlor	2.5	23	2	6	6	0	92
Spray							
Aldrin	.5	18	3	—	—	4	91
Heptachlor	.3	9	1	—	—	4	95
Untreated	—	89	77	100	74	93	—
L.S.D. at 5% level		11	9	3.5	12	5	

[a] Based on rows 3 feet apart.
[b] Maximum damage index (King & Forbes 1954), 100.

Appraisal Methods.—Final appraisal was based on data taken in the field at time of harvest. A sample of either 40 (Victoria, Courtenay, Agassiz, Puyallup) or 50 (Kelowna) rutabagas was taken from each plot, except in a few cases where there were fewer than the required number of roots in the plot—in which case all roots available were taken as the sample. All data were scaled to a basis of 40 roots per plot. Each rutabaga of the sample was examined and assigned to one of the four categories: clean, light, moderate, and severe (King & Forbes 1954). To these categories were assigned the numerical factors 0, 0, 2, and 4, respectively, selected as being approximately proportional to the economic severity of the attack. In general, roots in the severe category could not be marketed commercially as table grade, while those in the moderate

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[4] The authors gratefully acknowledge assistance throughout the experiments from other members of the staff of each laboratory. Mr. L. L. Stitt, formerly Entomologist at the Western Washington Experiment Station, took an active part in developing the common plan for the 1953 experiments.

[5] Aldrin was used as a 2.5% dust and as an emulsifiable concentrate containing 2 pounds of toxicant per gallon and was obtained from Shell Chemical Corporation, Denver, Colorado, and from Ansell Laboratories, Vernon, B.C.

DDT as a 5% dust and wettable powder, 50%, was obtained from Ansell Laboratories.

Dieldrin was used as a 2.5% dust and a wettable powder, 50%, obtained from Shell Chemical Corporation.

Heptachlor was supplied by Velsicol Corporation, Chicago, Illinois, as a 2.5% dust, as a 2.5% granulated material, and as an emulsifiable concentrate containing 2 pounds of toxicant per gallon.

Isodrin was used as a 2% dust obtained from Shell Chemical Corporation.

category could be marketed only after special trimming. Uniform appraisal of the damage and control in all five experiments was ensured by the close definition of categories and by the participation of the senior author in taking the harvest data at each place.

The use of a numerical factor for each category permitted the data for each plot to be combined into a single figure, the damage index (King & Forbes 1954).

Postgermination observations for phytotoxicity were made in all experiments, but only the data from Kelowna were suitable for statistical appraisal. These data consisted of counts of seedlings in each plot 10 days after germination commenced. Yields of rutabagas were also determined at Kelowna. The significance of all results was tested by an analysis of variance.

RESULTS.—Data for each experiment, expressed as mean damage indexes, are summarized in table 1. Results of treatments tested at only one locality are not tabled but are given in the text.

Phytotoxicity and yield data for the Kelowna experiment are given in table 2.

Several of the treatments gave excellent commercial control. Aldrin and heptachlor applied by the band or the furrow method gave satisfactory control in all five localities. However, phytotoxicity was evident with the furrow treatments at Courtenay and particularly at Agassiz and Kelowna. Spray treatments with aldrin and heptachlor gave very satisfactory control at Victoria, Courtenay, and Puyallup. These treatments were not tested at either Agassiz or Kelowna.

The granulated heptachlor formulation used did not show any promise. An isodrin furrow treatment of 2.5 pounds of toxicant per acre at Puyallup and seven foliage applications of dieldrin spray totalling 3.5 pounds of toxicant per acre at Kelowna gave very good results, reducing maggot damage by 90 per cent and 97 per cent, respectively.

Treatments tested at Kelowna that did not give satisfactory protection were: DDT furrow treatment at 5 pounds of toxicant per acre, DDT foliage spray at a total of 14 pounds of toxicant per acre in seven applications, dieldrin furrow treatment at 1.5 pounds of toxicant per acre, and dieldrin seed treatment at 0.16 pounds of toxicant per acre.

The data on seedling emergence for the Kelowna experiment (Table 2) show significant phytotoxic effects in all furrow treatments. This phytotoxicity is also reflected by the average yield of rutabagas per replicate. The yield data also show that, even where there was no phytotoxic effect, good control of root maggots did not significantly increase the *total* yield of rutabagas but only that of marketable roots.

DISCUSSION.—The results of all five experiments strongly confirm previous findings (King & Forbes 1954) and extend them to a wider range of conditions. They show that highly effective control of root maggots attacking rutabagas is feasible and economical with heptachlor and aldrin provided that the toxicants are present in adequate amounts, by residual action or by renewal, in the surface inch of soil next to the roots *throughout* the long period of time during which they need to be protected from infestation. The rate per acre depends on the method of application, the insecticide, and the spacing of rows.

Table 2.—Emergence and yields of rutabagas at Kelowna, 1953.

Method and Chemical	Pounds Toxicant, per Acre	Number of Seedlings, Percentage of Check	Weight of Roots (Pounds)	
			Total, Percentage of Check	Marketable, Per Cent
Untreated	—	100	100	27
Band				
Heptachlor	5	107	101	91[b]
Aldrin	5	70	80	70[b]
Foliage spray				
Dieldrin	3.5	96	82	68[b]
DDT	14	70	88	51[b]
Furrow				
Heptachlor	2.5	37[a]	58[a]	47
Aldrin	2.5	14[a]	38[a]	28
Dieldrin	1.5	12[a]	40[a]	16
DDT	5	40[a]	58[a]	11
Seed Treatment				
Dieldrin	0.16	60[a]	80	18

[a] Significant reduction.
[b] Significant increase.

Rates as given apply to rows 3 feet apart; if the rows are less than 3 feet apart, a proportionately greater amount per acre must be used so that the same amount of insecticide is applied per foot of row. Broadcast applications are not economical because of the relatively large amount of insecticide required.

Foliage spray applications of dieldrin directed to kill the adult flies before oviposition can take place may have some promise, provided that the cost of making the relatively large number of applications (e.g., seven at Kelowna) is not excessive. This type of treatment may hold promise where spraying of large acreages by aircraft is feasible.

Data appraisal.—Statistical appraisal of the results is based on the *damage index*, as study (King & Forbes 1954) had shown it to be the most accurate and sensitive measure of the damage caused by maggots and hence of the practical effectiveness of the chemical treatments. Phytotoxicity must also be considered in the case of furrow treatments.

Phytotoxicity.—The 1953 experiments showed clearly that there is a considerable risk of phytotoxic effects from the furrow method of insecticide application. Phytotoxicity was apparent in three of the experiments but resulted in a serious reduction of stand in only two of them. No phytotoxic effects were noted in the remaining two experiments. The most marked phytotoxicity occurred at Kelowna, where the insecticide was applied in direct contact with the seed, a rod-row seeder being used. In the other experiments, where a Planet Junior seeder was used, there was no phytotoxicity apparent or, if present, it was not severe. This difference was thought to be due to the effect of the Planet Junior foot in mixing a small amount of soil with the seed as it was being dropped into the opened furrow, which already contained the insecticide. Direct contact between the seed and the insecticide was thus reduced.

Aldrin was somewhat more phytotoxic to rutabagas than heptachlor. The Kelowna counts (Table 2) show these differences. At Agassiz, the aldrin furrow treatment showed marked phytotoxic effects but the similar heptachlor treatment showed little effect.

Observation indicated that there are several factors involved in the occurrence and severity of phytotoxicity. These factors are the amount of soil mixed with the insecticide and seed, the soil moisture, and the chemical. More careful experiments, including greenhouse trials, will have to be made to clarify this problem.

Provided that the problem of phytotoxicity can be solved, the furrow method is one of the best methods of treatment from the standpoint of effectiveness and especially on the basis of the relatively small amount of insecticide needed to give protection from the maggots.

Aldrin vs. heptachlor.—Table 1 gives 10 pairs of mean damage indexes for aldrin and heptachlor used by identical method and rate (furrow, 2.5 pounds; band, 5 pounds) in each of five localities; and 4 pairs (the two methods at each of two localities) were provided by the 1952 experiments (King & Forbes 1954). For these 14 pairs the average index of damage was 12.1 for aldrin and 7.2 for heptachlor, an average difference of 4.9 in favor of the latter. Though this difference was small in comparison with the maximum damage index of 100 and the average of 78 for the untreated checks in the seven experiments, it was significant at the 1 per cent level. Similarly Stitt (1953), in working with root maggots in turnips, found that "less injury occurred in the heptachlor treatments than in the aldrin treatments."

For the spray treatment, heptachlor at the 3-pound rate gave results that were not significantly different from those with aldrin at the 5-pound rate, in experiments at three localities in 1953 (Table 1) and two localities in 1952 (King & Forbes 1954). For these 5 pairs the average index of damage was 7.8 for aldrin and 5.8 for heptachlor.

Since both aldrin and heptachlor have been shown to be highly effective against this pest, however, the choice, if any, between them must be based mainly on economic and other considerations.

Granulated formulations.—Though the granulated heptachlor formulation tested in three of the experiments in 1953 was ideal from the standpoint of ease of application, the results obtained were unsatisfactory; they did not compare favorably with those obtained by a similar application of heptachlor dust, being little better than for the untreated check. Communication with Velsicol Corporation indicated that the particular lot of attaclay used in formulating this granulated preparation may have acted on the toxicant, causing it to break down. Analyses made by them showed that only 0.05 per cent heptachlor remained in a similar 2.5 per cent granulated formulation after 8 to 10 months in storage.

The authors emphasize, however, that until experimental proof is secured it is unsafe to assume that any granulated formulation is capable of providing the same effectiveness against root maggots in rutabagas as has been demonstrated for dust formulations. The known habits of the pest suggest the possibility that with the granulated formulation the distribution of the toxicant in the soil may be restricted, and perhaps its release delayed, to an extent that permits more of the newly hatched maggots to move to the roots without coming in contact with lethal amounts of the toxicant.

Effect of soil type.—Damage to rutabagas receiving one of the better treatments was consistently, and often considerably, heavier in the experiment at Victoria than in any of the others, even though the untreated rutabagas in the same experiment were not as severely damaged as were those at either Agassiz or Puyallup. The soil in part of the field used for the Victoria experiment was peaty with a high organic content, and the replicates therein showed greatly reduced control, whereas in the replicates in sandy loam soil the effectiveness of the treatments was closely similar to that obtained in the other four experiments.

Rutabagas vs. turnips.—Stitt (1953) obtained adequate control of root maggots in turnips by the use of aldrin or heptachlor by the furrow method at rates about one-fifth the furrow rate used for these chemicals in the present experiments with rutabagas. Earlier work of King & Forbes (1954) and an unpublished experiment (52–28) conducted in 1952 at Western Washington Experiment Station at Puyallup by L. L. Stitt (personal communication) showed that lower rates were inadequate for root maggot control in rutabagas. In the latter, Stitt obtained only 45 per cent control of root maggots in rutabagas with aldrin and 55 per cent with heptachlor by the furrow method at rates, respectively, of 0.100 and 0.092 pounds of toxicant per 1000 lineal feet of row (equivalent to 1.46 and 1.34 pounds per acre when rows are spaced 3 feet apart). In contrast, in his experiment 52–7 with turnips, Stitt (1953) obtained 88 per cent control with aldrin and 89 per cent with heptachlor by the furrow method at the much lower rates of 0.029 and 0.028 pound per 1000 feet of row (0.42 and 0.41 pound per acre for rows 3 feet apart).

In both experiments the hazard from root maggots was very severe, the mean damage index for the untreated checks being 97 for the rutabagas (52–28) and 93 for the turnips (52–7). Moreover, the experiments, though at different times (52–28) planted July 11, 1952, harvest date November 18; 52–7 planted April 15, 1952, harvest date July 30), were conducted at seasonal periods typical for each crop. Consequently, the results may be regarded as substantially representative and comparable.

It is thus clear that these two crops, though closely related both horticulturally and in their susceptibility to damage by root maggots, differ very markedly in the rates required for satisfactory control of these pests, with several insecticides. The much longer growing period of rutabagas, with the consequently greater exposure to this hazard, is no doubt a major factor.

SUMMARY.—Replicated experiments under a common plan were carried out in 1953 at Victoria, Courtenay, Agassiz, and Kelowna, British Columbia, and Puyallup, Washington. The results, confirming previous findings, showed that highly effective control of root maggots attacking rutabagas is possible and economical under a wide range of conditions, with heptachlor and aldrin. By the *band method*, 1 pound of toxicant per 3000 feet of row was applied as a 2.5 per cent dust raked in 1 inch in a 10-inch band, down the center of which the row was then seeded. By the *spray method*, two applications were made: the first just after an early thinning, the second as late as the crop growth permitted the use of power sprayers; at each application there was applied, per 3000 feet of row, 0.3 pound of heptachlor or 0.5 pound of aldrin, in about 40 gallons of water. The *furrow method*, by which 2.5 per

cent dust was placed in the planting furrow with the seed, was effective at half the rate used for the band method, but resulted in serious reduction of stand in two of the experiments. Where rows are 3 feet apart, the above rates equal 5, 3, and 2.5 pounds of toxicant per acre for heptachlor, and 5, 5, and 2.5 pounds for aldrin by the band, spray (total of the two applications), and furrow methods, respectively.

Seed treatments have not shown promise for the control of root maggots attacking rutabagas.

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Biological Studies of Peach Catfacing Insects in Illinois[1]

S. C. CHANDLER[2]

Peach catfacing is here defined as an early injury to the fruit, checks the growth at that point and results in a distorted and frequently lopsided peach, with a lack of pubescence in the area affected. Such injuries are shown in figure 1.

Biological studies of peach catfacing were begun in Illinois in 1925. In 1925, 1926, and 1927 studies were made simultaneously at Carbondale, Illinois, and at Vincennes, Indiana. It was found that the injury was caused usually by sucking insects, principally the tarnished plant bug, *Lygus lineolarius* (P. de B.), and several stink bugs, chiefly of the genus *Euschistus*, and to a somewhat lesser degree by the plum curculio *Conotrachelus nenuphar* (Hbst.). The flower thrips, *Frankliniella tritici* (Fitch), which at that time was thought to be the principal if not the only cause of the injury, was shown not to cause catfacing (Porter *et al.* 1928).

In 1944 jarring records were made in eight Illinois peach orchards to determine occurrence and relative abundance of catfacing insects at successive stages in the development of the fruit. Tarnished plant bugs appeared in early bloom or late pink, reached a peak in late bloom, declined rapidly after petal fall, and did not occur after shuck off. Stink bugs were first jarred in early bloom, reached a peak at shuck off, and were found in only small numbers thereafter, except for a time in July when a second brood was produced. Plum curculios were first found in late bloom, reached a peak about 1 week after shuck off, and were found in moderate numbers throughout the season (Chandler 1944).

In 1953 and 1954 jarring records were made in 26 orchards as a further check on the 1944 findings. These jarring records are summarized in table 1.

Table 1 shows that the tarnished plant bug was the first insect to appear, occurring first in the pink stage, reaching a peak in the late bloom and declining rapidly in numbers after that time, although a few were jarred as late as the shuck-off period. This seems to fit in with earlier findings that tarnished plant bugs are attracted to the blossoms and are found in only small numbers after all blossoms are off. It will be seen that the other important group of catfacing insects, stink bugs, were found first in early bloom, but did not reach a peak until shuck off, after which they decreased rapidly in numbers. They were found in small numbers until jarring was ceased. Due mostly to excessively hot, dry weather during the seasons of 1952, 1953, and 1954, the number of plum curculios jarred was very small. From the records in table 1 it appears that they were first jarred in full bloom, reached a peak when shucks were splitting and continued until at least 2 weeks after shucks were off. Thus, the pattern which was reported in 1944 was in general substantiated by the data collected 10 years later.

Table 1.—Numbers of insects jarred at successive stages in the development of the peach in 26 orchards in 1953 and 1954.

STAGES	PLUM CURCULIO	TARNISHED PLANT BUGS	STINK BUGS
Pink	0	7	0
Early bloom	0	15	5
Full bloom	1	39	0
Late bloom	6	66	11
Petals all fallen	5	17	21
Shucks splitting	7	7	39
Shucks half off	0	0	31
Shucks all off	5	4	131
1 week after shucks off	6	0	29
2 weeks after shucks off	4	0	8

CAGE TESTS.—In 1953 and 1954 tests were carried on with insects on trees near Carbondale, designed to secure information on the type of injury produced by the different insects concerned, their relative importance, and the stages of fruit development during which each worked.

Insects were introduced into screen wire cages about 18 inches long and 12 inchs in diameter, fitted with 18 inch cloth sleeves. The cages enclosed branches on the tree which had from 5 to 15 blossoms or fruits. The insects used in the tests were largely *Lygus lineolarius* and various stink bugs collected by jarring orchards or sweeping in nearby alfalfa. The principal stink bugs used in tests, determined by Dr. M. W. Sanderson of the Illinois Natural History Survey, were the following: *Euschistus servus; E. variolarius; E. tristigmus; Podisus maculaventrus* (*Say*); and *Thyanta custator*.

In addition the arboreal stink bug, *Brochymena quadripustulata*, was kept separate in cage tests to determine whether or not it produced the typical catfacing injury. Usually from 15 to 20 tarnished plant bugs were placed in each cage. Stink bugs were harder to secure, but in most cases 10 bugs were introduced into each cage. In

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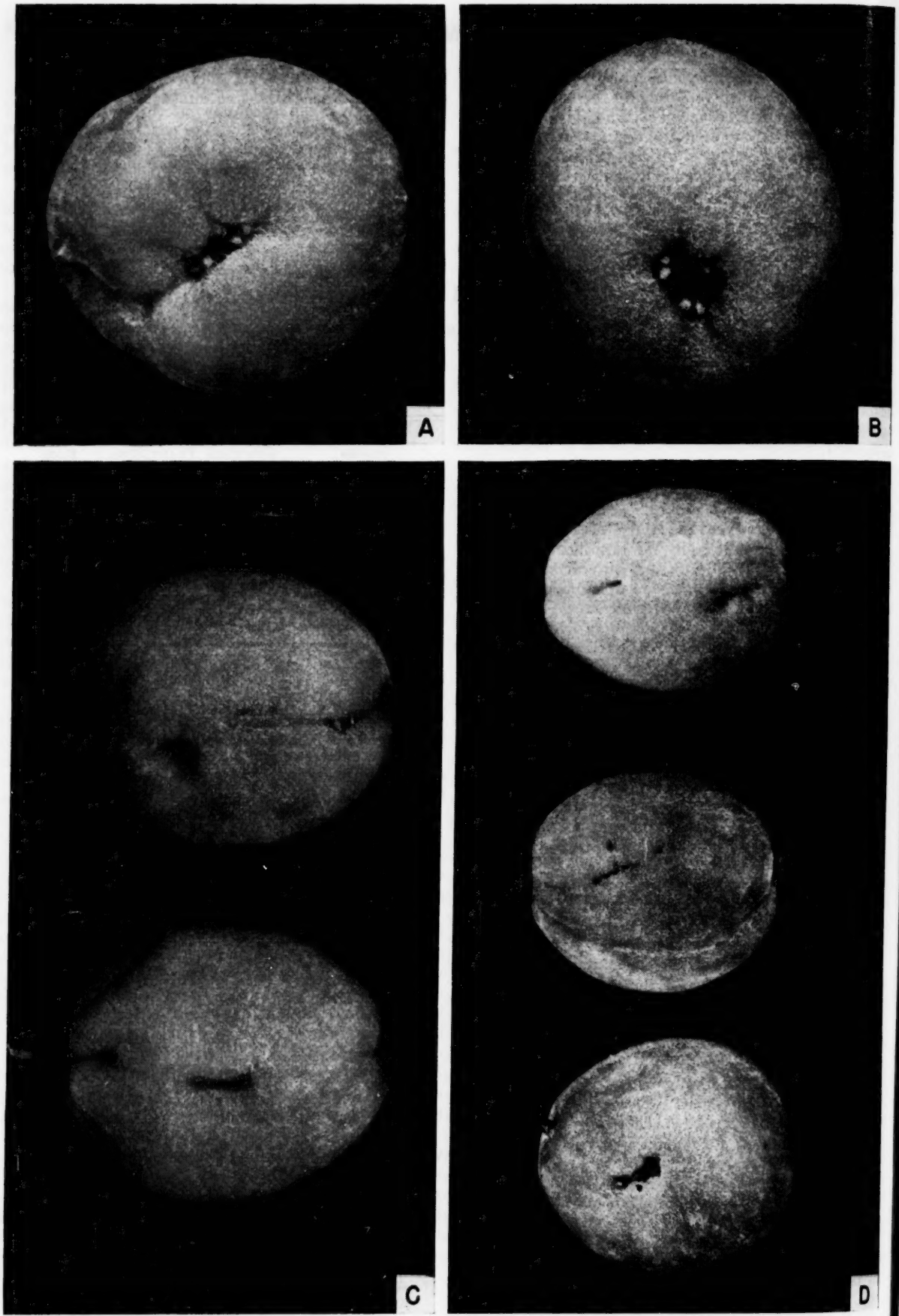

FIG. 1—Typical peach malformations commonly referred to as catfacing produced by: (A) tarnished plant bugs, (B) stink bugs, (C) plum curculio, and (D) needle pricks in young fruit.

some cages young peaches were also pricked with a needle to study mechanical injury. These cage tests for 1953 are summarized in table 2, and for 1954 in table 3.

Table 2.—Tests of peach catfacing in 1953 to determine stage in fruit development during which the injury occurs.

Stage of Development	Number and Condition of Peaches in Cages at Time of Examinations When Subjected to					
	Lygus		Stink Bugs		Needle Prick	
	Number		Number		Number	
	fruit	cat-faced	fruit	cat-faced	fruit	cat-faced
Bloom only	3	3				
Petal fall	3	3				
Bloom through shuck crack	2	2	6	6		
Bloom through shuck off			24	0		
Shuck crack through shuck off	3	2	14	7		
Shucks ¾ off					21	4
Fruit enlarged to ¾ inch diam.					50	0
7–15 days after shuck off	5	3	7	2		
18–22 days after shuck off	6	5	6	0		
25–29 days after shuck off	6	0	8	0		
31–39 days after shuck off	6	0	6	0		

In 1954 insects were left in the cages for a shorter period in an effort to determine more accurately the exact stage when injury takes place. These data are summarized in table 3.

Table 3.—Tests of peach catfacing in 1954 to determine stage of fruit development during which the injury occurs.

Stage of Development	Number and Condition of Peaches in Cages at Time of Examinations When Subjected to							
	Lygus		Stink Bugs		Plum Curculio		Needle Prick	
	Number		Number		Number		Number	
	fruit	cat-faced	fruit	cat-faced	fruit	cat-faced	fruit	cat-faced
Pink	2	0						
45% blossoms open	29	0	11	0				
Petal fall	1	0	10	3	6	3		
Shuck crack	4	3	3	2	6	3	19	2
Shuck off	5	0	6	3			9	4
1 week after shuck off	7	3	1	0				
2 weeks after shuck off	11	0	1	0				
3 weeks after shuck off	9	0						

Both in 1953 and in 1954 *Brochymena quadripustulata* bugs were introduced into cages continuously from petal fall through shuck off but no catfacing resulted.

The small number of peaches left in the cages in some cases is due to the natural drop of bloom and fruit and also to the feeding of the insects.

Reviewing the studies of both years it appears from tables 2 and 3 that catfacing may be produced as early as the blooming period by tarnished plant bugs, and that from the shuck-crack stage until shucks are all off tarnished plant bugs, stink bugs, and plum curculios, as well as pricks from a needle may produce the injury. However, it would appear that stink bugs are likely to be responsible for most of the injury during the shuck-crack to shuck-off period, especially since jarring has shown that tarnished plant bugs rapidly decrease after petal fall and stink bugs increase. Information on the latest date of possible catface injury differed somewhat in 1953 and 1954. Table 2 shows injury as late as 18 to 22 days after shucks are off, whereas table 3 shows none after 1 week. Examination of the extent of the injury shows that only small injured areas were found on fruit exposed to insects in cages later than the first week after shuck off. It will be noted that there is a limit to the time when pricking with a needle will produce catfacing. It is probable that the artificial injury should be considered similar to that caused by the plum curculio. Catfacing by this insect, Fig. 1C, is smaller in area than that caused by the tarnished plant bugs and stink bugs shown in Figs. 1A and B. It is quite probable that the sucking insects inject something into the peach which may not be done by the plum curculio and certainly not by a needle prick, Fig. 1D, which is similar to plum curculio injury, Fig. 1C.

SUMMARY.—Plum curculio, *Conotrachelus nenuphar* (Hbst.), the tarnished plant bug, *Lygus lineolarius* (P. de B.), and a number of stink bugs are responsible for most of the injury known as catfacing in Illinois peach orchards. Injury caused by the tarnished plant bug is indistinguishable from that made by the stink bugs. That caused by the plum curculio is similar to that caused by the other two species, but usually is smaller in area. For two seasons the arboreal stink bug, *Brochymena quadripustulata*, did not produce catfacing in cages. Needle pricks in young fruit produced injury similar to that made by the plum curculio.

Typical injury may be produced by insects as early as the blooming period and as late as 3 weeks after shucks are off, but most of it is produced by injuries made between the petal fall and shuck-off stages.

Jarring in 26 orchards showed that tarnished plant bugs were the first catfacing insects of the season to appear, being found during the pink stage of peach with peak occurrence in late bloom or petal fall. Stink bugs appeared first in early bloom, with peak occurrence at petal fall or shuck split. Plum curculios appeared first at full bloom with a peak at shuck off.

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SCIENTIFIC NOTES

Aphodius pardalis Lec.[1] A New Turf Pest[2]

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In April, 1954, grub damage to golf fairways at Eugene, Oregon, was called to the attention of the writers by Mr. Howard, greenskeeper of the Eugene Country Club. He indicated that some 125 to 150 acres of turf were affected. Grubs found beneath the injured sod were identified as *Aphodius* spp.

The writers visited the Eugene Country Club April 28 and found many, small, bluish, *Aphodius* larvae in the soil near or underneath scattered, brown patches of turf. Some of the injured turf could be rolled up like a carpet and the feeding of the grubs was very evident. Grub abundance varied greatly throughout the fairways, but in some instances populations of from 240 to 644 larvae per square foot were observed. An adjoining public golf course, where there was less irrigation and fertility, appeared to be uninfested.

On April 28, about half of the grubs were just below the sod; others were in cells ¼ inch to 3 inches deep. All larvae appeared to be nearly full-grown. By May 10, the turf was recovering from grub attack, following heavy watering, and much of the injured turf was putting out new roots. In some areas most of the *Aphodius* larvae had moved downward in the soil to pupate. Pupae were first observed on June 4 and the first new adults were found on June 14. The depth of pupation ranged from 1 to 7 inches.

Reared adults were submitted to O. L. Cartwright of the U. S. National Museum who identified them as *Aphodius pardalis* Lec. and reported that the U.S.N.M. collection contains specimens of this species from Ashland, Corvallis, Hood River, and the Willamette Valley (all in Oregon) and a single specimen from Kamloops, B. C. Leng (1920) lists the species from Southern California and Vancouver.

Other instances of damage to turf by aphodian larvae have been recorded in the literature. *Aphodius howitti* Hope is injurious to pasture in Australia and Tasmania (Given, 1950) and *Aphodius pseudotasmaniae* Given causes extensive damage in Tasmania (Martyn, 1950). Hoffman (1935) published an account of *Ataenius cognatus* (Lec.), a pest of golf greens in Minnesota. It is probable that other species of *Aphodius* and allied genera are also pests of turf or growing crops but have been largely ignored because of the general impression by text book writers and others that aphodians are only dung feeders.

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[1] Coleoptera: Scarabaeidae.

[2] Accepted for publication February 9, 1955.

A New Leafhopper Attacking Prunes in California[1]

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The prune leafhopper, *Typhlocyba prunicola* Edwds, is a common pest of prunes in California, but it was not recognized until Oman (1948) first recorded it from prunes in California. Heagele (1930) had previously reported the species from prunes in Oregon and Idaho. Since the biology of this species was not known, studies were begun in California during the latter part of 1952.

During the course of the investigation in the 1953 season, another closely related species was found to be infesting prune orchards. The species was determined as *Typhlocyba quercus* (Fab.)[2] a pest that had not been reported from the United States up to that time. Ross and De Long (1949) however, had recorded it from Queen Anne Cherry in British Columbia.

A search of the literature revealed that although considerable taxonomic work had been done with the species in Europe, the biology had not been studied. Therefore, the biology of this new insect pest was investigated along with the prune leafhopper. The portion of the life history that was missed during the 1953 season was investigated in 1954. Although *Typhlocyba quercus* has not been given a common name in the literature thus far, it seems logical to propose the name, oak leafhopper.

Typhlocyba quercus was found to have one generation a year and the majority of the overwintering eggs were laid under the bark of 2- to 5-year-old wood from June to August. There are five nymphal instars and the nymphs of each instar are quite active.

Under California conditions, French prune is the preferred host of both leafhopper species. In some orchards the populations of *T. quercus* were observed to be higher than those of *T. prunicola*, or vice versa, while in others the populations were maintained at equal level. Imperial prune is not a favored host of either species, and population levels are relatively low on this host. Sweet cherry was also observed to be a host of *T. quercus* in the field, but the populations were below the economic level. However, injury to the leaves of sweet cherry, due to feeding of this insect, is rather severe. The leaves turn pale yellow and necrotic areas develop between the veins. Sometimes, if the feeding is not extensive, only stippling of the leaves may occur.

The egg parasite, *Anagrus epos* Gir., parasitized a few eggs of the oak leafhopper, but was more effective against the prune leafhopper. Another egg parasite, *Anagrus armatus nigriceps* Gir., which was reared from the eggs of the latter species, did not seem to parasitize the eggs of *T. quercus*.

The nymphal and adult population peaks of *T. quercus* follow 3 to 4 weeks after that of *T. prunicola*. This as stated by Madsen (1954) complicates the control picture to some extent. Consideration of the life history of these two species is necessary in order to develop a control program effective against both insects.

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Alfalfa Pollen on Nectar-Collecting Honey Bees[1]

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Honey bees, *Apis mellifera* L., collecting pollen from alfalfa have been shown to carry pollen on the corbicula as well as on the face or posterior portion of the head (Vansell & Todd 1946). Woodrow (1952) and Minderhoud (1954) mentioned another characteristic mass of pollen on the bee, which occurs ventrally in that posterior portion of the head (fossa) normally covered by the retracted mouth parts (Fig. 1). Until recently it has not been known that the nectar-collecting bee on alfalfa also carries this mass of pollen.

FIG. 1.—Posterior view of the decapitated head of a honey bee that collected nectar from alfalfa. The pollen appears as a white mass, lower center. The mouth parts are pulled down by a metal hook.

All pollen-collecting honey bees taken for examination in Arizona alfalfa fields during August 1954 also had this fossal pollen, but none of those from cotton, Johnson grass, pigweed, and puncturevine out of flight range from alfalfa fields.

In July 1954 observations were made on honey bees collecting nectar from alfalfa at Davis, Calif. All the blossom visitors in samples taken at different times of day from 9 a.m. to 3 p.m. carried this pollen. Some of the bees caught on yellow star-thistle, birdsfoot trefoil, or lima bean blossoms in the vicinity of alfalfa fields also had the characteristic accumulation of alfalfa pollen in their fossae. This indicates that every honey bee collecting alfalfa nectar trips some flowers and, further, that the bee is unable to readily remove the pollen mass.

The presence of this alfalfa pollen even on the earliest morning visitors indicates that some intercrossing may be expected in alfalfa varieties grown anywhere within flight range of a colony of honey bees.

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Effect on Beneficial Insects of Three Insecticide Mixtures Applied for Cotton-Insect Control in 1954[1]

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Experiments to determine the effect on beneficial insects and spiders of insecticides applied for the control of cotton insects were begun in 1953 (Gaines 1954). In a continuation of this work in 1954 tests were made with three dust mixtures—toxaphene plus sulfur, dieldrin plus DDT and sulfur, and BHC plus DDT and sulfur. Eight applications were made from July 15 to August 24, the first six by airplane and the last two with a tractor duster. The mixtures were applied at the average rate of 10.9 pounds per acre-application in two fields and 12.6 pounds in a third field.

The effect on beneficial insects was determined by examining the plants on 25 row-feet and by making 100 sweeps with a 16-inch net at four points in each field. The results, summarized in table 1, show that after the third application the beneficial insect and spider populations were practically eliminated in all fields.

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[2] The field work was performed by J. W. Deaton and B. J. Smittle.

Table 1.—Effect of three insecticide dusts on beneficial insects and spiders in cotton fields.[a]

Date[b] (Week Beginning)	Lady Beetles	Flower Bugs	Lacewings	*Geocoris*	Assassin Bugs	Spiders	Syrphids
	Field 1 (62 acres)—Toxaphene 20%+Sulfur 40%						
June 14	4–	3–	0–	0–	3–	6–	0–
21	15–5	28–1	0–1	0–1	21–5	13–2	0–0
28	38–9	83–9	5–1	13–2	15–7	31–5	0–0
July 6	29–4	179–18	8–1	34–6	30–3	47–8	5–1
12	33–6	190–29	6–1	29–9	39–4	45–9	1–0
19	23–2	11–4	10–2	5–2	8–1	22–1	0–0
26	16–4	2–1	7–4	1–1	1–1	13–1	1–1
Aug. 2	19–6	0–0	1–1	0–1	1–1	8–1	0–0
9	0–2	0–0	2–0	0–0	1–0	1–1	1–0
16	6–6	0–0	1–0	0–0	0–0	3–0	0–0
	Field 2 (76 acres)—Dieldrin 1.5%+DDT 5%+Sulfur 40%						
June 14	8–	0–	0–	0–	1–	8–	0–
21	18–7	9–1	0–0	2–1	7–4	16–3	0–0
28	20–10	68–13	3–0	11–3	16–3	24–4	0–0
July 6	32–6	181–12	5–0	23–3	21–2	38–5	0–0
12	58–13	163–26	6–2	33–13	22–5	44–11	2–1
19	70–5	23–5	12–2	14–3	22–1	17–3	1–0
26	72–9	9–4	4–3	6–1	7–1	10–1	1–0
Aug. 2	25–13	0–2	3–2	0–1	1–1	3–2	1–0
9	2–1	0–0	0–0	0–0	0–1	2–2	1–0
16	3–2	1–0	0–0	0–2	0–0	0–1	0–0
	Field 3 (73 acres)—BHC, gamma 3%+DDT 5%+Sulfur 40%						
June 14	9–	1–	0–	0–	1–	5–	0–
21	18–6	24–2	1–0	5–1	11–6	14–3	0–0
28	47–7	170–14	4–1	16–3	21–2	20–4	0–0
July 6	44–2	205–30	12–1	24–4	24–5	24–6	5–1
12	74–13	109–24	7–1	20–12	21–4	26–7	0–1
19	74–6	27–10	6–4	8–4	13–1	19–5	1–1
26	61–8	4–1	7–2	2–0	8–1	8–3	0–1
Aug. 2	4–2	4–0	0–1	0–0	1–1	1–1	0–0
9	1–1	0–0	2–0	0–0	0–0	4–0	0–0
16	0–0	0–0	0–0	0–0	0–0	1–0	0–0

[a] First figure is the number per 100 feet of row and second figure the number per 100 sweeps of a net.

[b] Counts made through July 12 were pre-application counts.

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Ips confusus (Lec.) Adults Infected with Nematodes[1]

A. D. MOORE,[2] *California Forest and Range Experiment Station, Forest Service, U.S.D.A.*[3]

In recent studies on the toxicity of organic insecticides to the California five-spined engraver beetle, *Ips confusus* (Lec.), many of the adults used as test subjects were found to be infected with two different species of nematodes. Specimens of these were identified by Dr. M. W. Allen, Department of Nematology, University of California, as *Aphelenchulus* sp. (near *reversus*) and *Aphelenchoides* sp.

Natural mortality was relatively high among the test subjects used in the toxicity studies, and it was felt that the nematode infections may have been responsible for this mortality. Therefore, all of the untreated check beetles that died during the toxicity studies (total of 74) and 100 of the untreated checks that lived were examined to determine the incidence of nematode infection in each group. The beetles were sexed as they were examined to determine the rate of infection by sex. The results were as follows:

Twenty-one per cent of all beetles examined were infected with *Aphelenchulus* sp. and 83 per cent with *Aphelenchoides* sp. Most of the beetles infected with *Aphelenchulus* were also infected with *Aphenenchoides*.

The *Aphelenchulus* were in the adult stage, and from 1 to 12 individuals were found free within the body cavity of each infected beetle. All of the *Aphelenchoides* were in an immature stage and were usually found in groups of 10 to more than 100, either in the rectum, free in the abdominal cavity, or under the elytra.

The percentage of infected beetles by sex in the checks that died during the toxicity observations and in the checks that survived was as follows:

	Dead beetles		*Surviving beetles*	
Species of nematode	*Males*	*Females*	*Males*	*Females*
Aphelenchulus sp.	18	17	32	18
Aphelenchoides sp.	77	73	88	90

The data indicated that there was no correlation between mortality among the check beetles and nematode infections, and no appreciable difference in infection between sexes. Therefore, it was concluded that the infections could be disregarded in analyzing the results of the toxicity studies, and the observations were discontinued.

[1] Accepted for publication March 14, 1955.

[2] Presently Assistant Specialist, Department of Entomology and Parasitology, University of California, Berkeley. Collaborator, CFRES, Berkeley.

[3] The California Forest and Range Experiment Station is maintained by the Forest Service at Berkeley, California, in cooperation with the University of California.

A Mechanical Insect Collector with High Maneuverability[1]

W. C. KENNARD and J. L. SPENCER, *Federal Experiment Station, Mayaguez, Puerto Rico*

A study of pollination in the tropical fruit, mango (*Mangifera indica* L.), made collections of the insects associated with the inflorescences necessary. Hand nets and other usual methods of trapping (Peterson 1937, Oman & Cushman 1946) proved unsatisfactory. Difficulties were experienced not only with the actual capturing of insects but also with the separation of small forms from plant debris. Observations revealed that some of the smaller types, *e.g.*, thrips, were not appearing in the collections with a frequency suggested by their numbers on the flowers. In order to overcome these problems and provide greater efficiency, a mechanical collecting device which creates suction through dual separating nets was constructed.

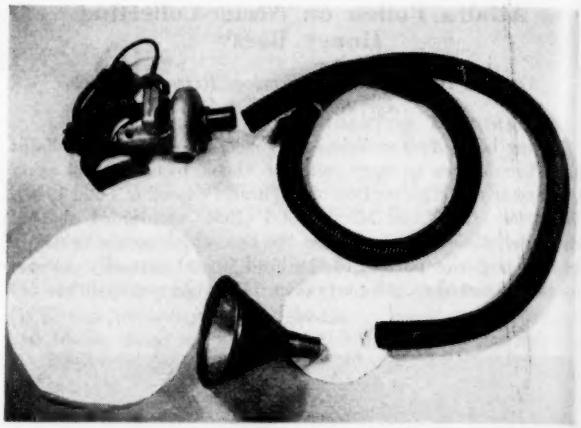

FIG. 1.—Components of collector. Clockwise from upper left: vacuum unit showing carrying strap, flexible air hose, fine-mesh net, 10-inch funnel, and coarse-mesh net.

This collector is an adaptation of a hand-type vacuum cleaner[2] fitted with a maneuverable collecting device (Fig. 1). The intake of the blower unit was modified to receive a flexible air hose with an inside diameter of 2 inches. Into the other end of the hose was fitted the collecting unit consisting of two nets and a collecting cone or funnel. The net of finer weave, a section 10 inches in diameter of very small mesh nylon, was placed in the hose and secured by means of rubber bands to the lip. This was followed by the delivery end of a 10-inch funnel pressed into the hose against the net. A second net of coarser mesh with an 18-inch diameter was placed in the funnel and fastened to the edge with a large rubber band. The addition of a shoulder strap to the blower and a light-weight pole fastened to the funnel end resulted in a very satisfactory portable collector. A toggle switch on the motor handle permitted local control. The entire device, including pole, weighed only 14½ pounds.

This device enabled the operator to collect within a considerable vertical as well as horizontal range without resorting to ladders (Fig. 2). Collections were made easily to a height of 20 feet and greater distance could have been achieved by lengthening the hose. The insects were retained within the nets by continued operation of the motor, and then were stunned by wafting an open bottle of ethyl acetate over the outer net. The motor was turned off, and the large insects on the coarse net were separated from the debris and dropped into a killing jar containing potassium cyanide. The inner, fine-mesh net with adhering insects was then removed from the air hose and placed in another killing jar. This method provided undamaged specimens of the immature forms and small types for low-power microscopic examination. Contact injury, so often noted in hand net collecting, was not apparent and its absence was attributed to the immobilization of the captured insects by the constant suction of this device. In situations where an unusual amount of debris was present, a disk of hardware cloth with a diameter the same as the

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[1] Accepted for publication June 13, 1955.

[2] Martindale Blow-Er-Clean, Model B, manufactured by the Martindale Electric Co., Cleveland 7, Ohio.

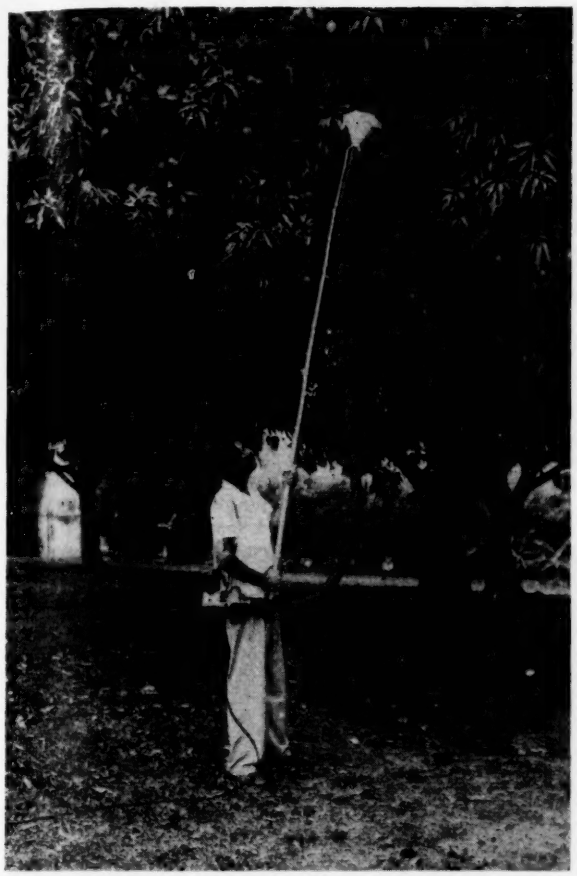

FIG. 2.—Collector in operation sampling insect fauna in mango orchard.

interior dimension of the funnel lip was placed in the intake of the apparatus.

The trees from which collections were made in this experiment were close enough to a building to allow the use of commercial electrical power. Power could be supplied by a portable, 110-volt generator, or a 6-volt unit could be operated from a vehicle battery. The use of a long extension cord would allow adequate freedom for sampling several trees without relocating the power source.

With this light-weight apparatus, large numbers of insects can be collected in a limited period of time with a minimum of effort. By substituting funnels of other dimensions at the collecting or intake end, this collector may be directed to capture insects from a specific location. The employment of multiple nets not only permits a rough separation of forms according to size while the collector is in operation, but the outer net also excludes much of the debris. The constant suction on the captured insects reduces contact injury to a minimum, and the pole and flexible hose provide for considerable maneuverability.

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A Device for Sub-slab Pressure Injection of Insecticides[1]

JOHN V. OSMUN and DAVID C. PFENDLER, *Purdue University, Lafayette, Indiana*

One of the problems pertaining to the application of insecticides, fumigants, and other economic poisons as well as certain beneficial soil chemicals, is the insertion and dispersion of such materials in the soil beneath concrete slabs and other solid barriers placed over earth. Among the current needs which exist is that in termite control operations where soil beneath slab floors, porches, steps and sidewalks must be chemically protected or fumigated. Penetration of such soil by chemicals is often inhibited by a combination of soil type, porosity, organic content, and moisture level, making application difficult or virtually impossible.

An injection unit, called a sub-slab nozzle, has been developed permitting pressure injection and sealed delivery of liquids and gasses. This nozzle is attached to the hose line of a conventional spray apparatus or gas cylinder. Although the extent of chemical dispersion in the earth is dependent on soil factors mentioned, very substantial penetration and lateral spread has been experienced in several soil types where other methods of introduction have failed.

Figure 1 illustrates the device. The nozzle assembly consists of

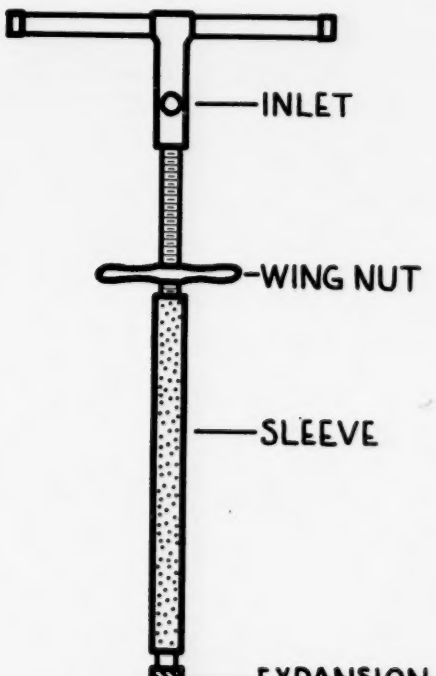

FIG. 1.—Sub-slab injection nozzle.

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[1] Accepted for publication June 9, 1955.

a steel tube around which is fitted a short flexible expansion collar of pliable and expandible material such as rubber or plastic. The collar is supported by a removable sleeve against which the collar may be pressed by lever action above. The latter is activated by a screw assembly or similarly functioning device which pushes down upon the upper ring of the expansion collar. To operate, the nozzle assembly is inserted into a hole slightly larger than the outside diameter of the expansion collar, drilled through a slab or other barrier. After insertion, the collar is expanded against the sides of the hole by adjusting the screw assembly. This locking action prevents the withdrawal of the nozzle during operation and premits either high pressure injection of liquids or introduction of fumigants without gas leakage. The nozzle is removed by releasing the stress against the expansion collar. Patent proceedings on this device are being pursued.

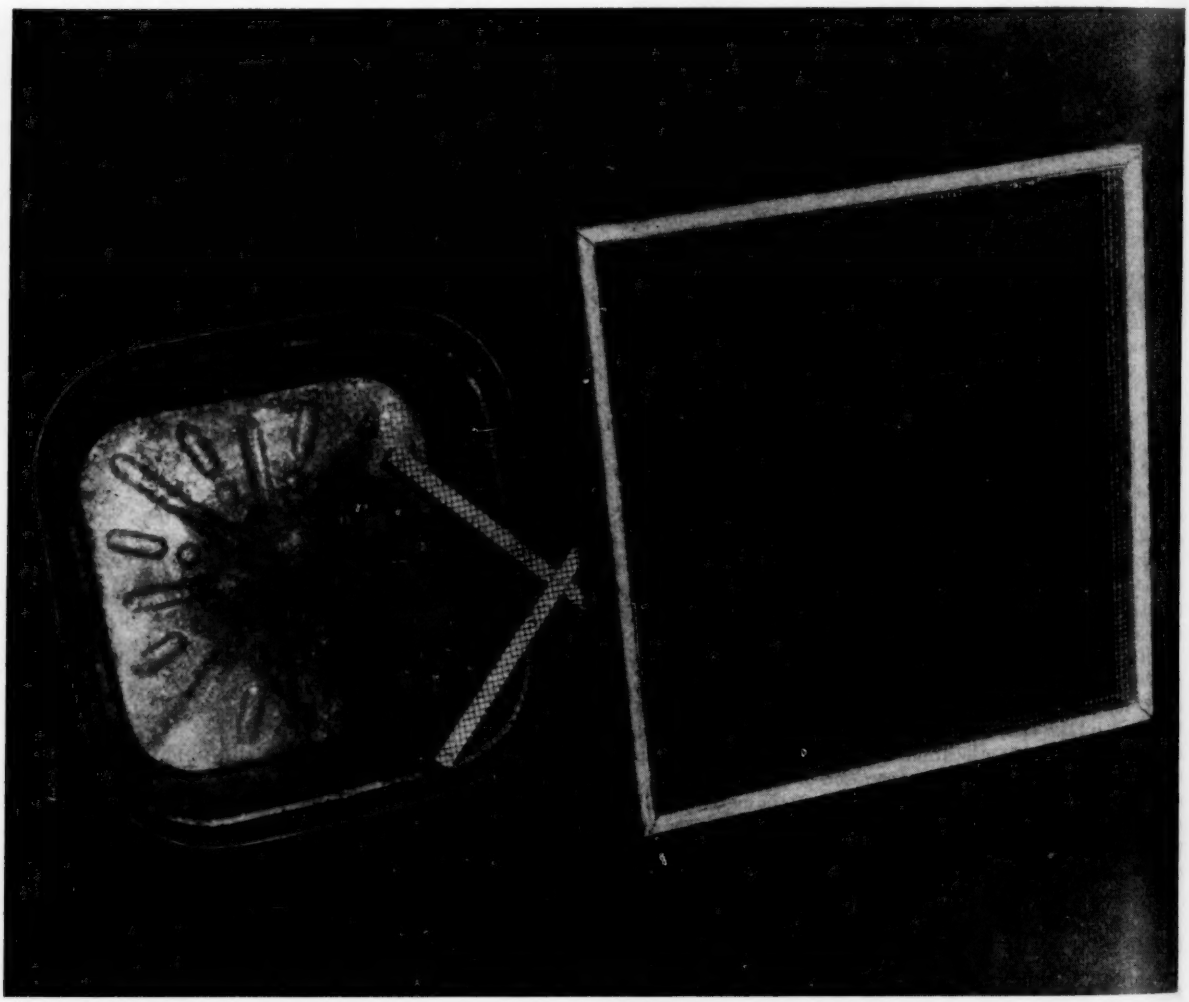
A Convenient Method for Rearing Large Cockroaches[1]

PAUL A. DAHM, *Department of Zoology and Entomology, Iowa State College, Ames*

Cockroaches are reared in various ways for use in insect physiology and toxicology. Piquett & Fales (1952) have summarized the literature pertinent to and described their methods for rearing several species of cockroaches, including the American and German cockroaches (*Periplaneta americana* (L.) and *Blattella germanica* (L.)).

This note describes a convenient and an economical procedure for rearing the American cockroach, the Madeira cockroach (*Leucophaea maderae* (Fabricius)), and the giant cockroach (*Blaberus giganteus* (L.)). American cockroaches, being indigenous, were obtained from local collections. The Madeira cock-

[1] Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1256. Supported in part by a Grant-in-Aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council. Accepted for publication March 16, 1955.



A

FIG. 1.—Galvanized tub cockroach rearing unit. (A) Expanded view of tub, wire flooring, and screen cover. (B) American cockroach culture, showing tub, wire flooring, drinking water fountain in center, inverted cylindrical one-quart cardboard containers, dishes of dog food, and sections of apples.

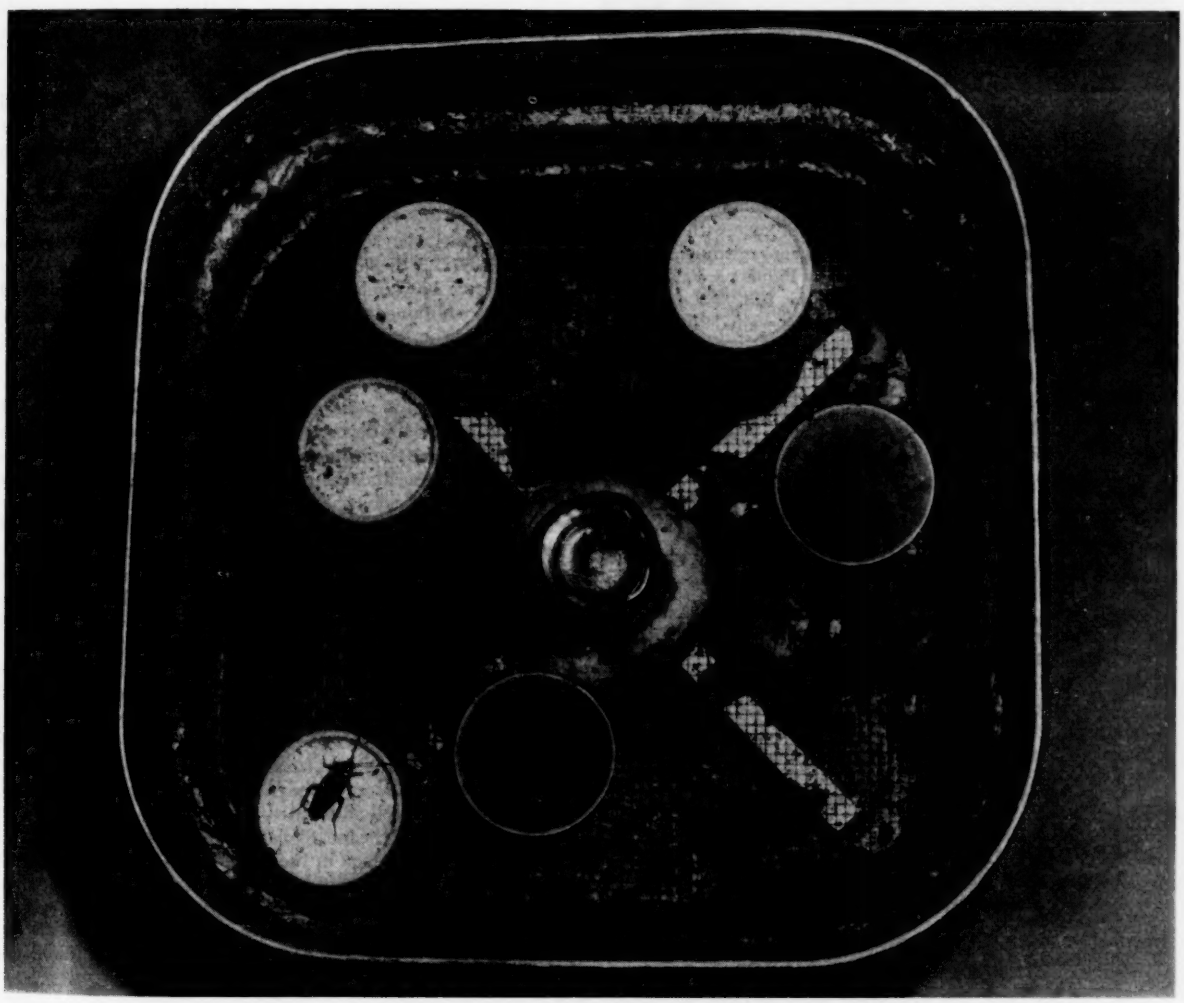
roach cultures were developed from a few specimens obtained from Dr. Berta Scharrer[2]; Rehn (1945) has described the distribution of, and Gurney (1953) the distribution, general bionomics, and recognition characters of this cockroach. The latter paper is especially useful in differentiating between the two sexes of the Madeira cockroach. Scharrer (1951, 1953) has described rearing methods and dissection techniques with the Madeira cockroach in the process of using it for experimental tumor induction. The cultures of *Blaberus giganteus* were obtained from A. C. Miller.[3] Cockroaches from which this culture was started were determined by J. A. G. Rehn of the Academy of Natural Sciences, Philadelphia, Pa. This large cockroach, according to Rehn, is widely distributed in tropical South and Central America.

A rather simple procedure has been developed for rearing these three species of cockroaches. The basic rearing unit is a square, galvanized, metal washtub (Figures 1A & 1B); these tubs are available commercially, the ones we use are about 20½″ square and 11″ deep. A loosely-fitting, false flooring is made by stapling a piece of ¼-inch galvanized wire onto painted wooden cross-pieces as shown in figure 1A. This wire flooring is raised an inch or two above the bottom of the tub, and supports a drinking water fountain,[4] containers of dog food, and inverted, cylindrical, 1-quart cardboard containers in which the cockroaches can hide. Sections of apples or other fruit or vegetables are spread over the wire flooring on a weekly basis. The loosely-fitting wire flooring allows roaches to crawl beneath it at will. A thin layer of sawdust is spread on the bottom of the tub before placing the sub-flooring into the tub. Debris falls through the wire flooring and collects on this sawdust layer where it can be removed readily with the sawdust. In the case of American cockroach cultures, the sawdust also serves as a convenient deposition place for oothecae. A typical arrangement for a culture of American cockroaches is shown in figure 1B.

A thin band of petrolatum about 2 inches wide is spread around the inside top of the tub. This discourages most of the cockroaches from escaping. A cover is also placed over each tub (Figure 1A). This cover is made of 1-inch lumber strips about 2 inches wide, nailed and screwed at the corners and covered with both a copper screen (16-mesh to the inch) and ¼-inch galvanized wire. The latter is added primarily to protect the wire screen. A half-round molding is added to cover the sharp edges of the screen and wire.

A series of these culture tubs can be handled conveniently on an angle-iron rack as shown in figure 2. This rack is equipped with rubber-covered, swivel casters and can be moved around in the rearing room or laboratory. Tags for identifying and dat-

[2] Dept. of Anatomy, Univ. of Colorado, School of Medicine, Denver, Colo.
[3] Gulf Research & Development Co., P. O. Drawer 2038, Pittsburgh 30, Pa.
[4] Glass, poultry drinking fountains; No. 6P, Brower Mfg. Co., Quincy, Ill.



B

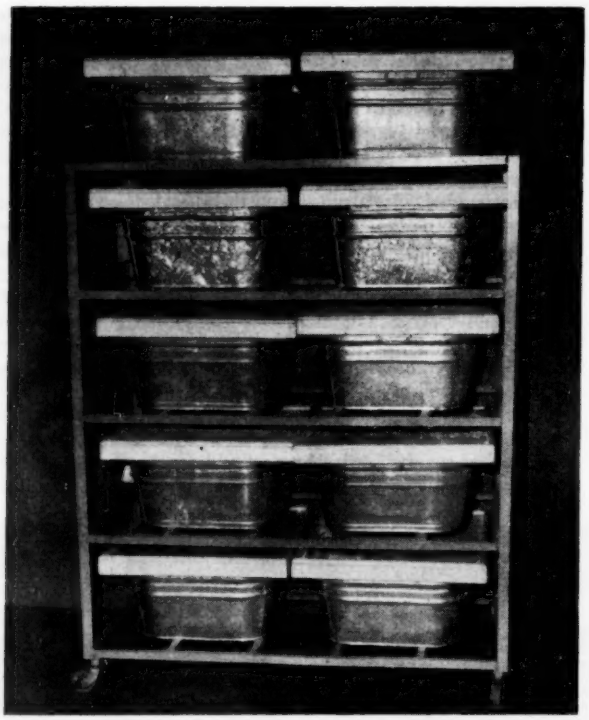


FIG. 2.—Cockroach rearing units on angle-iron rack.

ing the cultures can be attached to the tub handles. From 500 to 1000 adult American cockroaches can be accommodated easily in one tub; the number of cockroaches will vary with the number of quart cartons used. To prevent over-crowding, we have tried to employ the formula suggested by Piquett & Fales (1952) for an optimum cockroach population per tub.

Cultures of the three species of cockroaches cited above generally need care only once a week. At that time, fresh water is added, the cellulose cotton used in the watering fountains replaced if dirty, more dog food added, and sections of apples added. Additional time is needed, of course, to establish new cultures.

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Residual Effectiveness of Mixtures of Organic Phosphorus Insecticides with Chlorinated Terphenyls[1]

IRWIN HORNSTEIN, WILLIAM N. SULLIVAN, and CHING HSI TSAO,
Entomology Research Branch, Agr. Res. Serv., U.S.D.A.

The authors have reported (1953 a, b, c) that the residual effectiveness of relatively volatile chlorinated hydrocarbon insecticides such as lindane, aldrin, and heptachlor can be increased by the addition of an isomeric mixture of chlorinated terphenyls,[2] and have described suitable methods of applying these mixtures (1954, 1955). In this paper mixtures of chlorinated terphenyls and several volatile organic phosphorus insecticides are evaluated for their residual effectiveness

In preliminary tests the chlorinated terphenyls were found to be good solvents for these common organic phosphorus insecticides.

The insecticides used in these tests were parathion, Diazinon, malathion, Bayer L 13/59, dehydrochlorinated L 13/59 (Mattson *et al.* 1954) shown to be dimethyl 2,2-dichlorovinylphosphate (Barthel *et al.* 1954), and diethyl 2,2-dichlorovinylphosphate.

Methyl ethyl ketone solutions of the chlorinated terphenyls and the insecticide, separately and together, were prepared. Concentrations were so adjusted that 10 ml. of the final solution uniformly distributed on 8×10-inch glass plates gave a deposit of 25 mg. of insecticide together with 0, 25, or 100 mg. of chlorinated terphenyls per square foot. The treated plates were stored vertically in a rack in a partially opened hood with the fan continuously running to insure adequate ventilation. The indoor temperatures during the test period ranged from 65° to 85° F.

The test insects were 2- to 3-day-old house flies (*Musca domestica* L.), large-nymph and adult cockroaches (*Periplaneta americana* L.), and confused flour beetles (*Tribolium confusum* L.). The insects were exposed to the treated plates by confining them under petri dishes for 60 minutes, and then transferred to separate cages and supplied with food. Mortality counts were made after 24 hours for flies and after 4 days for roaches and flour beetles.

The results of these tests are given in table 1.

The addition of chlorinated terphenyls increased the residual effectiveness of all the insecticides. Except for malathion, longer lasting residues were obtained with the 1:4 ratio of insecticide to chlorinated terphenyls than with the 1:1 ratio.

The results obtained with dimethyl 2,2-dichlorovinylphosphate and its ethyl homolog were impressive. Alone neither was of any appreciable value after 2 hours. However, a 1:4 mixture of dimethyl 2,2-dichlorovinylphosphate and chlorinated terphenyls gave 100% mortality after 60 days, and almost as good results were obtained with the ethyl homolog. In addition to the high mortalities, unusually quick knockdowns were observed, particularly with the dimethyl 2,2-dichlorovinylphosphate.

The effectiveness of an insecticide deposit containing chlorinated terphenyls is maintained by diffusion of fresh toxicant from the body of the deposit to the surface (Hornstein & Sullivan 1953). The lower the vapor pressure of the insecticide the more slowly it is replaced at the surface, and in turn the greater the fraction of insecticide that may be rendered unavailable by decomposition. If with a given amount of insecticide the amount of chlorinated terphenyls is progressively increased, at some dilution the vapor pressure and surface concentration of the insecticide will drop to a point where the material is no longer effective. This dilution value will be a function of the vapor pressure of the pure material and its inherent toxicity.

In this study 1:4 malathion-chlorinated terphenyl deposits were ineffective. Malathion has the lowest vapor pressure and the least toxicity of the compounds tested.

Conversely, the higher the vapor pressure of the insecticide and the greater its inherent toxicity the more marked will be the increase in effectiveness of the insecticide-chlorinated terphenyl residue. This is well illustrated by the two chlorovinylphosphates, which were the most volatile compounds tested and were comparable to parathion in their toxicity to insects.

In some preliminary outdoor tests malathion and Diazinon, alone and in combination with chlorinated terphenyls, were applied to pine foliage for residue tests against house flies. The results paralleled those found later in our indoor tests. Malathion

[1] Accepted for publication February 3, 1955.

[2] Available commercially as Aroclor 5460 from Monsanto Chemical Co., St. Louis, Mo.

Table 1.—**Residual effects against house flies, cockroaches, and flour beetles of some organic phosphorus insecticides in combination with chlorinated terphenyls.**[a]

Chlorinated Terphenyls (Mg./Sq. Ft.)	Per Cent Kill of Residues of Indicated Age													
	House Flies					American Cockroaches					Confused Flour Beetles			
	2 Hours	4–5 Days	10 Days	30 Days	60 Days	2 Hours	4–5 Days	10 Days	30 Days	60 Days	4–5 Days	10 Days	30 Days	60 Days
						Parathion								
0	100	100	100	7	—	—	100	100	0	—	100	9	5	—
25	100	100	100	100	—	—	100	100	48	—	100	100	0	—
100	100	100	100	100	—	—	100	100	97	—	100	100	88	—
						Diazinon								
0	100	12	6	—	—	100	0	0	—	—	2	—	—	—
25	100	98	67	33	—	100	2	3	6	—	8	3	0	—
100	100	100	100	100	—	96	26	4	35	—	22	3	0	—
						Malathion								
0	100	100	50	17	—	79	70	31	11	—	100	3	0	—
25	100	100	100	4	—	90	60	21	11	—	100	87	2	—
100	37	19	0	—	—	37	0	8	—	—	15	18	4	—
						Bayer L 13/59								
0	100	12	16	—	—	—	100	39	0	—	0	0	0	—
25	100	100	60	12	—	—	100	65	10	—	0	0	2	—
100	100	100	97	12	—	—	100	100	21	—	20	32	2	—
						Dimethyl 2,2-dichlorovinyl phosphate[b]								
0	50	2	0	—	—	—	0	—	—	—	0	—	—	—
25	100	100	100	100	100	—	100	100	78	19	100	100	100	0
100	100	100	100	100	100	—	100	100	97	100	100	100	100	100
						Diethyl 2,2-dichlorovinyl phosphate[b]								
0	33	2	0	—	—	—	0	—	—	—	0	—	—	—
25	100	100	100	100	100	—	100	100	60	30	100	100	5	0
100	100	99	100	100	100	—	100	100	94	100	100	100	6	24

[a] Deposits on glass plates aged at 65° to 85° F. indoors. All insecticides applied at 25 mg./sq. ft. All tests replicated at least twice.
[b] W. F. Barthel, B. H. Alexander, Paul A. Giang, and S. A. Hall, chemists of the Entomology Research Branch, prepared these compounds.

plus chlorinated terphenyls in a 1:4 ratio was less effective than malathion alone. Diazinon alone and with equal quantities of chlorinated terphenyls were ineffective after 4 days, but when used in a 1:4 ratio the mixtures gave 92% kill after 30 days and 77% after 60 days.

SUMMARY.—The addition of chlorinated terphenyls to volatile organic phosphorus insecticides prolonged the insecticidal effectiveness of the residues. Diazinon and Bayer L 13/59 were materially improved. Outstanding results were obtained with dimethyl 2,2-dichlorovinylphosphate and its ethyl homolog compounds that are extremely toxic to insects but have too high a vapor pressure for residual treatments.

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Ethylene Dibromide and Methyl Bromide Fumigation of Apples Infested with Apple Maggots[1]

HENRY H. RICHARDSON,[2] *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

Ethylene dibromide fumigation was reported to be highly effective against the oriental fruit fly, *Dacus dorsalis* Hendel, and the melon fly, *Dacus cucurbitae* Coq., in Hawaii in 1951 (Balock, 1951, Balock & Lindgren 1951). It was subsequently used in the quarantine treatment of fruits and vegetables infested with these insects, as well as the Mediterranean fruit fly, *Ceratitis capitata* (Wied.). Since then this fumigant has also been found effective against the West Indian fruit fly, *Anastrepha mombinpraeoptans* Sein, in mangoes and *A. suspensa* (Loew) in almendras

[1] Accepted for publication March 14, 1955.
[2] Acknowledgment is made with thanks to R. W. Dean, New York Agricultural Experiment Station, for furnishing maggot-infested apples; to R. H. Foote, of the Entomology Research Branch, for identification of the fruit fly; and to Herbert Roth, D. Kamper, L. F. Byars, and M. A. McMasters, of the Plant Quarantine Branch at Hoboken, N. J. for occasional assistance in carrying on parts of this work.

(Richardson 1952), as well as the Mexican fruit fly, *A. ludens* (Loew), in mangoes (Shaw & Lopez 1954).

In the fall of 1953 small-scale tests were made at Hoboken, New Jersey, to determine whether ethylene dibromide was also effective against another fruit fly, the apple maggot, *Rhagoletis pomonella* (Walsh), infesting apples. Methyl bromide had previously been indicated to be effective against this insect (McLaine & Monro 1937, Monro 1938, Chapman 1940), and tests were also made with this gas.

Twelve fumigations were made at normal atmospheric pressure and temperatures near 70° to 87° F. in several 27- and 50-cubic-foot steel chambers. Two-hour exposures and continuous fan circulation were used in all tests. Approximately 1400 apples of three varieties (Wealthy, Greening, and McIntosh), involving about 3350 maggots, were used. Fruit loads varied from about 5 to 20 per cent of chamber capacity. Ethylene dibromide was vaporized on a hot plate inside the chamber, adjusted so that vaporization was complete within 5 minutes. Methyl bromide was vaporized in the usual way by immersing the injection tube in hot water. Methyl bromide concentrations were checked in most fumigations by means of thermal conductivity analyses. Fumigated and untreated apples were kept in emergence cages for about 50 days at approximately 75 to 85° F., and examinations were usually made for pupae—after larval emergence from apples—at weekly or shorter intervals. In addition, samples of the fruit were cut by hand and examined for live or dead larvae about 7 and 50 days after each fumigation. The number of pupae from untreated apples was 2.90 per apple for Greening, 2.78 for Wealthy, and 0.23 for McIntosh.

With ethylene dibromide a dosage of $\frac{1}{4}$ pound per 1000 cubic feet for 2 hours gave complete kill in three tests at temperatures near 70 to 76° F. In a fumigation at $\frac{1}{8}$ pound per 1000 cubic feet at 87° there was some survival—98.6 per cent estimated mortality of 432 larvae. At these dosages no effect from fumigation could be seen on the fruit. However, Claypool & Vines (1953) have shown that apples will tolerate $\frac{1}{2}$ pound and sometimes higher dosages of ethylene dibromide.

With methyl bromide there was complete kill in five tests at dosages of $1\frac{1}{4}$ to 2 pounds per 1000 cubic feet at about 70° F. and also in two tests at 1 pound and $1\frac{1}{4}$ pounds at 87°. The only survival was in a test at $\frac{5}{8}$ pound at 87°, in which there was a 99.6 per cent estimated mortality of 324 larvae.

These small-scale tests indicate that ethylene dibromide has a high efficiency against the apple maggot, similar to that against other fruit flies. Methyl bromide had considerable efficiency, as found by previous workers, but appeared to be somewhat less effective than ethylene dibromide.

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A Technique for Estimating the Percentage of Honey Bees Visiting Alfalfa[1]

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As early as 1947 members of the Legume Seed Research Laboratory at Logan, Utah, observed that most honey bees, *Apis mellifera* L., working in alfalfa fields had a small accumulation of alfalfa pollen in the proboscis fossa, or underside of the neck region (Grout 1949). In 1950 G. E. Bohart observed that in Alberta, Canada, where tripping by nectar collectors is rarely seen, all 18 bees he collected from an alfalfa field had alfalfa pollen in the proboscis fossa. Vansell (1955) also observed fossal pollen in California. In 1952 some observations were initiated to determine the importance of these deposits of pollen.

In order to establish just how many bees visiting alfalfa had this deposit in the proboscis fossa, 158 bees working in several alfalfa fields in northern Utah were examined. Fossal pollen was found on 91 per cent of them. Microscopic examination confirmed that this was alfalfa pollen.[3] Pollen is probably deposited in the proboscis fossa by the release of the staminal column when blossoms of these plants are tripped by the bee. Apparently she is unable to clean off all the pollen from this location and a sizeable lump accumulates (Fig. 1). When a bee is no longer working alfalfa blossoms, the lump becomes smaller, more compact, and in a few days loses the bright yellow color of the fresh accumulation (Fig. 2).

Field bees collected at hive entrances were also examined. One series of samples was collected in June from colonies located near the foothills in North Logan, Utah, where many sources of nectar and pollen were available. Another series was collected in August from colonies in seed fields where alfalfa was the predominant forage plant for bees in the area. The data obtained from these samples are summarized in table 1.

Table 1.—Occurrence of fossal pollen on honey bees collected at hive entrances, Cache Valley, Utah, 1952.

PERIOD	LOCATION OF APIARY	NUMBER OF BEES EXAMINED	PER CENT OF BEES WITH FOSSAL POLLEN	PER CENT OF FOSSAL POLLEN THAT WAS ALFALFA
June	Foothills	241	34	92.4
August	Seed field	410	51	99.9

Practically all the fossal pollen found on the bees located in the seed fields was alfalfa pollen. A slightly larger per cent of the fossal pollen from the bees near the foothills was not alfalfa pollen. Clover, vetch, and mustard were other pollens sometimes

[1] Accepted for publication March 15, 1955.

[2] In cooperation with the Utah Agricultural Experiment Station.

[3] Woodrow (1952) and Minderhoud (1954) report that pollen accumulations in this location are found on bees working red clover (*Trifolium pratense*) and white clover (*T. repens*).

FIG. 1.—Alfalfa pollen in proboscis fossa of honey bee, behind lorum.

FIG. 2.—Proboscis fossa of honey bee showing bare membrane behind lorum, with no pollen accumulation.

found in the proboscis fossa, unmixed or mixed with alfalfa. Often the shape of these accumulations was different from that of alfalfa pollen, in that the pollen was distributed along the tipites and mentum rather than in one compact lump.

It appeared that the occurrence of alfalfa pollen in the proboscis fossa might be used for estimating the percentage of field bees in the colony working in alfalfa fields. Therefore, the following experiment was carried out. A pair of colonies that had been in the mountains for about 1 month were moved to an alfalfa field, and a pair that had been in the alfalfa field at least 2 weeks were moved to the alfalfa-free mountain location. For 5 days, starting the day after the moves, samples were collected from the entrances of the colonies. Table 2 summarizes the data obtained from these samples.

It is clearly shown that the percentage of field bees with alfalfa pollen increased for 5 days when the bees were able to work alfalfa, but dropped to almost nothing when the bees were removed from alfalfa. Conditions beyond our control prevented the continuance of the sampling after 5 days.

Two possible sources of error are apparent when estimating from hive-entrance samples the percentage of field bees working alfalfa. Since table 1 shows that as many as 10 per cent of the bees in an alfalfa field may not have fossal pollen, this fact should be taken into consideration. It might be best to sample the bees in local fields to establish just what this correction factor should be. Also, since it can take up to 5 days for the fossal pollen to disappear from a bee after it leaves alfalfa, the percentage obtained from entrance sampling could be an overestimate of the actual percentage of bees working alfalfa. From what is known of the flower constancy of honey bees and their working habits, this error would probably be negligible except when the number of available alfalfa flowers is undergoing rapid reduction through being cut or maturing into seed. If these factors are taken into consideration, this technique should prove to be of considerable use.

Table 2.—Occurrence of fossal pollen on bees collected at entrances of hives in two locations, Cache Valley, Utah, 1952.

Date (July)	Number of Bees Collected		Per Cent of Bees with Alfalfa Pollen in Fossa	
	Mountain	Alfalfa Field	Mountain	Alfalfa Field
14	34	30	41	0
15	40	34	30	12
16	87	47	17	30
17	88	58	15	22
18	75	59	4	41

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Carbowax as a Carrier of Systemic Insecticides for Treating Wheat Seed[1]

EDMON J. KANTACK[2]

A technique for insecticidal treatment of wheat seed with systemic toxicants was developed during the summer of 1954, in connection with studies on the chemical control of the wheat curl mite, *Aceria tulipae* (K.), on young wheat plants. The process concerns translocation of the toxic materials through the plant system. Although these studies are not completed, it appears advisable to outline this technique at this time, together with a brief summary of results, because of its possible early use by other investigators on seed treatment studies involving systemic toxicants.

Techniques to devise a better means of bringing the systemic toxicants into contact with the young plant roots were studied. Carbowax 6000,[3] a water soluble polyethylene glycol with a melting point of 60 to 63° C., was selected as the most promising carrier after several materials, including Carbowax 4000, were tested.

In early attempts to find a suitable carrier, medicinal paraffin with a melting point of 54 to 56° C. was tried by heating the paraffin and adding the toxicant and a xylene solvent. When the seeds were coated, the distribution of the toxicant did not appear to remain uniform in the paraffin after xylene had volatilized. Furthermore, the rapid solidification of the paraffin-toxicant combination made the seed coating process difficult. Carbowax 4000, another water soluble polyethylene glycol differing from Carbowax 6000 in that it has a lower molecular weight and a melting point of 53 to 56° C., was then tried. The formulation was made by heating the wax in a water bath and adding quantities of the technical toxicant in the desired proportion while the carrier was in a liquid state. This appeared to result in a uniform blend of the toxicant and carrier. The seeds were kept constantly in motion while this formulation was poured over them. A uniform plastic-like coating of the formulation was obtained on the outer surface of the wheat kernels.

Germination tests were conducted at the State Seed Laboratory, Topeka, Kansas, and in soil tests at the Kansas Agricultural Experiment Station, Manhattan, on Pawnee wheat seed treated with various rates of Carbowax per bushel of seed as well as with similar amounts of toxicant-Carbowax treated seed. The toxicants used were technical grades of Chlorthion,[4] demeton,[4] Meta-Systox[4] (the dimethoxy-homolog of Systox), schradan,[5] Am. Cyanamid 12008,[6] and Am. Cyanamid 12009.[6]

Concentrations of Carbowax 4000 as high as 12 pounds per bushel gave no indication of reduction or retardation of germination. Reduction in germination was evident, however, in certain toxicant-carrier treated seed, and this percentage reduction was generally in proportion to the concentration of the toxicants. In trials where water was added to the formulation to insure an even distribution on the seed, reduction in germination appeared to be greater. Water also appeared to alter some of the properties of the Carbowax, resulting in a flaking-off of the material from the seeds. In general, good germination was obtained when ½ pound or less of the toxicants tested was blended with 4 pounds of Carbowax 6000 per bushel of wheat seed.

Carbowax 6000 was tried in the toxicant-carrier formulations when it was thought plants from seeds treated with Carbowax 4000 might have caused excessive wilting during extremely dry conditions. Studies were then conducted to determine how plants from Carbowax 6000 treated seed minus the toxicants might react to drought conditions. Plants from seed treated with 1, 2, 3 or 4 pounds of Carbowax 6000 per bushel were grown in the greenhouse. Five-inch flower pots containing 3 plants each were used. Plants from each of these four treatment rates plus untreated control plants were arranged in rows. Three hundred mls. of water were applied daily to the soil in each pot in the first row; on alternate days in pots in the second row; every 3 days in pots in the third row; every 4 days in pots in the fourth row, etc., up to and including the seventh and final row which was watered only each seventh day. No differences in appearance, such as wilting and color, were observed. After 7 weeks both the aerial and root portions were washed and then air dried 10 days at a constant room temperature of 70° F. Weight records of the plants were made of each individual series of treatments. The differences in total plant bulk of roots and aerial plant portions among the Carbowax treatments and check plants were non-significant when tested by an analysis of variance method.

Comparisons of this coating method of seed treatment with a soil drench method of applying the toxicant, using the six systemically active toxicants, were made in the greenhouse. Equal rates of a toxicant per acre by the two methods of application resulted in the seed treatment method generally giving the greater reduction in populations of wheat curl mites during the first 4½ weeks. In addition, the soil drench method did not appreciably reduce mite populations until 1 week after application, whereas, the Carbowax seed treatment method gave effective control almost immediately after plant emergence. However, the soil drench method had an advantage over seed treatment during periods of rapid plant growth, detectable at about the third week, probably because the expanding root systems could pick up more of the toxicant distributed through the soil. Based upon mite counts on the plants, the dilution factor of the toxicant in the plants appeared not to be so great in the soil drench method as in the seed treatment during this period of rapid plant growth.

Four and one-half weeks after application the greater reduction of mites was obtained on plants given the soil drench treatment, although mite populations for both methods of treatment were somewhat higher at this time when compared with those on check plants. Tests extended through 7 weeks after which none of the toxicants in the tests gave substantial reduction in mite populations by either method, although certain toxicants still gave some reduction in populations.

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[2] Acknowledgment is made to William E. Robbins for suggesting the use of Carbowaxes, and to Herbert Knutson, under whose direction various studies on chemical control of the wheat curl mite are being conducted.

[3] Carbowax purchased from Carbide and Carbon Chemical Corporation, St. Louis 8, Missouri.

[4] Supplied by Pittsburgh Coke and Chemical Company, Pittsburgh 25, Pa.

[5] Supplied by Monsanto Chemical Company, St. Louis 1, Mo.

[6] Supplied by American Cyanamid Company, Stamford, Conn.

Control of the Sweet Potatoweevil in Puerto Rico[1]

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The most serious impediment to the development of a commercial, large-scale sweet potato industry in Puerto Rico is the sweet potatoweevil, *Cylas formicarius elegantulus* (Sum.). Until a few months ago, sweet potato tubers originating in Puerto Rico could not legally be imported into the United States (Federal Horticultural Board Quarantine No. 30), although other fruits and vegetables from Puerto Rico entered under inspection and certification. Even for local consumption, the public was forced to accept the very poorest and most indigestible kind of tubers because they were least liable to be weevil-infested. The fine flavored, orange or pink fleshed, non-fibrous varieties of sweet

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potato which are most desirable for human food could not be grown commercially because they are so greatly preferred by the weevils. And of even the inferior varieties, infestation becomes more pronounced the longer they are left unharvested in the field: the subsistence farmer's primitive method of storage. Unpromising and indeed almost hopeless as the situation seemed, the need for additional commercial crops, to, in part at least, take the place of sugar-cane, made imperative the solution of this problem.

In the control of white grubs, *Phyllophaga* spp., lindane and aldrin are almost equally effective, but the toxicity of aldrin to the grubs of the leaf-eating weevil or "vaquita," *Diaprepes abbreviatus* L., is at least 20 times as great as that of BHC (Wolcott 1951). Indeed, numerous experiments elsewhere have shown aldrin to be especially toxic to many other kinds of weevils, and it seemed possible that it might prove toxic to the sweet potato-weevil. The real difficulty lay in discovering an effective method of application. The habits of *Cylas* are an almost perfect protection against the external application of an insecticide, for it passes the entire subterranean period of its life-cycle within an infested tuber.

Preliminary tests were made to determine if aldrin, or any other chemicals which might be used, had any appreciable deleterious effect on the growth of sweet potato plants. Thus tubers were planted on November 15, 1950 in cans filled with soil being retained for determining the residual effectiveness of insecticides (Wolcott 1954). These tests were hardly conclusive, for the tubers grew too vigorously to be retained in the comparatively small space of the cans, but at least no indication of toxicity to the plants was shown, even with aldrin at the rate of 100 pounds per acre. Successful experiments in Hawaii (Sherman 1951) in the control of other sweet potato pests implied the possibility of comparable results with *Cylas* in Puerto Rico.

On January 14, 1952, a plot of 12,000 sq. ft. at the Isabela Sub-Station was selected for tests. Three-quarters of the area was sprayed with 23% aldrin Equivalent Emulsifiable Concentrate at the rate of 3 lbs. per acre, the untreated quarter of the area being a check. On January 22nd, half of the area was planted with tops of the superior variety UPR 3 which had been submerged in a solution of aldrin (23%) in 50 gal. of water. The remainder of the area was planted with untreated tops. Unfortunately, these did not give a good stand, and were replaced with similarly treated tops on February 25th. By April 21st, the growth of vines had half covered the ground, and those on the far fourth of the area were sprayed with 25 gallons of water in which a pint of 25% emulsifiable aldrin had been added, followed by three additional sprays at monthly intervals.

On August 5th, harvesting was commenced. A selection at random of 20 large, 60 medium and 20 small tubers was made from the eight center rows of each of the four plots, and these were split lengthwise with a machette to determine insect infestation. Of 100 tubers from the check, only 38 were free from injury by the weevils; those from the sprayed soil had 61 clean tubers; those from the soil sprayed and tops submerged had 90 uninjured tubers, while of the 100 tubers from the plot in which the soil had been sprayed, the tops submerged and the vines sprayed four times, 98 were clean. That such a result could be obtained is all the more surprising because the area selected for the tests adjoined a much larger area from which a sweet potato variety experiment, heavily infested with weevils, had just been harvested. Unseasonable rainfall had delayed harvesting for $1\frac{1}{2}$ months, thus prolonging the period during which infestation and re-infestation might occur. Considering these unfavorable factors, in addition to the known high susceptibility of the variety UPR 3 to weevil attack, the result was a triumph in chemical pest control.

The tubers harvested from the plot with the three-fold treatment with aldrin had a fine appearance, and their consumption after cooking by all those connected with the tests, and all too numerous friends, showed not only no off-flavor due to the application of the insecticide, but rather the greatest delicacy of flavor and repeated requests for additional samples.

The average yield of sweet potatoes in the Isabela region of Puerto Rico is from 30 to 40 quintales per acre. The variety UPR 3 is not only most desirable and attractive to the consuming public, but its yield is considerably higher, and in the check plot of the experiment it produced 48.1 quintales. In the plot in which only the soil was sprayed, the tuber yield was 84.2 quintales: a result presumably as much due to the elimination of white grubs in the soil as to its effect on the weevil, for the plot with tops submerged yielded only 82.2 quintales. But the beneficial results of the three-fold treatment with aldrin (to soil, to tops and to vines) is most apparent in the yield of 112.1 quintales per acre: a result which should entirely justify the use of this insecticide merely for its effect on the yield, quite aside from its primarily intended effect in producing sound, weevil-free tubers.

To determine to how great an extent each factor in the aldrin application is essential for the control of *Cylas*, an additional experiment was initiated at Corozal 3 weeks after the first one at Isabela was harvested. In the heavier soil at Corozal, weevil infestation proved to be not as severe as at Isabela, in the two check plots being 17% and 14%. This experiment indicated, however, that each factor in the insecticide application was essential if complete elimination of the pest was to be obtained.

During the fiscal year 1953–54, Dr. Luis F. Martorell conducted an additional test at the Isabela Sub-Station to determine the relative effectiveness of DDT, dieldrin, aldrin, lindane and heptachlor. The autumn of 1953 was exceptionally rainy, and large numbers of the southern armyworm, *Prodenia eridania* Cramer, and hornworms of *Herse cingulata* F. attacked the leaves and even the stems of the sweet potato plants. It is possible that the one-third increase in the yield of tubers in the areas in which the vines were sprayed was largely due to this incidental prevention of caterpillar attack. Of the five insecticides tested, DDT and lindane were the least effective, while the difference between heptachlor, dieldrin and aldrin was very small. In no case did any give 100% control, doubtless due to the checkerboard arrangement of small plots, which would not occur in commercial fields. These experiments show that it is entirely possible to produce sweet potato tubers entirely free of weevils on a commercial scale, not only for canning and local consumption but also for export shipment.

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Laboratory Tests of Diazinon-Butoxy Polypropylene Glycol Residues[1]

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Because butoxy polypropylene glycol (Crag Fly Repellent) has shown promise as a synergist and extender or formulation

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Table 1.—Residual tests on plywood against house flies with Diazinon-butoxy polypropylene glycol (B.p.g.) formulations.

Formulation		Mg. Diazinon Applied per 100 Sq. In.	Days from Application to Test	Minutes of Exposure of Flies	Per Cent Mortality After 24 Hours
Per Cent Diazinon	Per Cent B.p.g.				
Water Emulsions[a]					
10	0	50	21	15	100
			77	10	100
			126	3	0
			203	5	11
10	0.5	50	21	15	100
			77	10	100
			126	3	99
			203	5	72
10	10	50	21	15	100
			77	10	100
			126	3	91
			203	5	81
10	30	50	21	15	100
			77	10	100
			126	3	79
			203	5	96
Acetone Solution					
1	0	0.75	1	5	100
			10	5	44
			35	5	0
			49	5	0
1	5	0.75	1	5	100
			10	5	99
			35	5	95
			49	5	70
1	10	0.75	1	5	100
			10	5	91
			35	5	82
			49	5	60
0.5	10	0.75	1	5	100
			10	5	26
			35	5	30
			49	5	17

[a] Emulsion concentrate contained 10% Diazinon, 8% Triton X-188, and 82 to 72% of xylene-methyl ethylketone mixture (2:1) with or without butoxy polypropylene glycol as indicated. A dilution of 1 part concentrate to 19 water was used.

aid for pyrethrins and other insecticides (Haynes & McKenzie 1955), it was thought desirable to determine whether it would improve the residual activity of Diazinon. Accordingly, water emulsion concentrate and acetone formulations containing 0.5 to 10% Diazinon and from 0.5 to 30% butoxy polypropylene glycol were applied to plywood panels and filter paper surfaces.

Laboratory reared 4 to 6 day old house flies, *Musca domestica* L., were confined on the surfaces for various times under cellophane hoop cages using the method originally described by Barber & Schmitt (1948). Mortality was determined 24 hours after exposure to the residue. Between tests the treated surfaces were stored in the laboratory at 72 to 75° F., protected from sunlight.

The results of tests with dilutions of water emulsion concentrates and acetone solutions pipetted on to plywood are shown in table 1. Results of acetone solution applications to filter paper are shown in table 2. In the initial tests with water emulsion dilutions, 50 mg. of the acetone ingredient, Diazinon, was effective for 77 days but was ineffective when tested 126 days after application to the plywood. When 0.5 to 30% butoxy polypropylene glycol was used with the same amount of Diazinon, the effectiveness of the residue was extended to over 200 days.

Table 2.—Residual tests on filter paper against house flies with Diazinon-butoxy polypropylene glycol acetone formulation.

Formulation		Mg. Diazinon per 100 Sq. In.	Days From Application to Test	Minutes of Exposure of Flies	Per Cent Mortality After 24 Hours
Diazinon	B.p.g.				
1	0	25	1	5	100
			7	5.5	0
			12	15	5
1	0.5	25	1	5	100
			7	5.5	6
			12	15	10
1	1	25	1	5	100
			7	5.5	24
			12	15	10
1	2.5	25	1	5	88
			7	5.5	46
			12	15	35
1	5	25	1	5	87
			7	5.5	33
			12	15	70
1	10	25	1	5	82
			7	5.5	22
			12	15	77

In order to accelerate the work, subsequent tests were made at lower concentrations and a smaller amount applied to the panels. These were acetone solutions and it was found that although the residue from 1% Diazinon alone at 0.75 mg. per panel was not toxic to house flies within a week after application, formulations containing this amount of Diazinon and 5 or 10% butoxy polypropylene glycol, continued to be effective for over 5 weeks. In order to further accelerate the testing, acetone solutions were applied to 12.5 cm. diameter filter papers. In these tests 1% Diazinon applied at the rate of 25 mg. per 100 sq. in. of paper was ineffective after 1 day whereas residues from formulations containing 2.5 to 10% butoxy polypropylene glycol showed definite residual action 12 days after application.

In these tests, applications of butoxy polypropylene glycol had no residual toxicity. It is realized that applications to different surfaces and under different conditions may result in varying degrees of effectiveness. Further laboratory and field studies with the Diazinon-butoxy polypropylene glycol formulations are planned in which it is hoped some light will also be thrown on the physical and chemical aspects of such mixtures.

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Erratum

Volume 48, No. 3, page 292, in the article by Charles A. Triplehorn in the list of host plants given in column 2 the heading, "Plants Refused in Laboratory Feeding Trials," should be inserted after Red-osier dogwood and before Corn (sweet). The last 22 host plants listed were refused by the Asiatic oak weevil in laboratory feeding trials.

EDITORIAL

Our Society's Responsibility to the Annual Review of Entomology

For more than a year publications of the Entomological Society of America have carried references to the forthcoming Annual Review of Entomology. Dr. Edward A. Steinhaus, editor of the Review, has given a full account in the June issue of the Bulletin of the E.S.A. It seems advisable to discuss the Review further, since some members of the Society may not realize the full extent and importance of the responsibilities toward it that the Society has shouldered. The Annual Review of Entomology gives promise of being such a valuable contribution to entomology that nothing should be spared to put across to members a full appreciation of the situation.

The committee of this Society that in 1953 supported the idea of an Annual Review of Entomology and those members who contributed to the final plans had in mind more than just another good book for our libraries. They visualized a book which would be sufficiently comprehensive and at the same time present up-to-the-minute information on advances of our science in such a readable manner that scientists in many allied fields—in fact, a significant portion of the general public—would read it. Here, they reasoned, is one way for entomologists to tell an intelligent audience what we have done and are doing. We will therefore be supporting an activity that probably will improve the status of entomology, when we order one of these valuable books for our individual use.

The Review will be published each year as a single bound volume of 400–450 pages, and will consist of about 21 articles on broadly representative aspects of entomology. Some of the articles in Volume I are as follows: Physiology and Biochemistry of Diapause; Mode of Action of Insecticides; Nonbiting Flies and Disease; Veterinary and Medical Acarology; Ecology of Forest Insects; Insecticides and Their Effect on the Natural Balance of Arthropod Populations; Insect Transmission of Plant Diseases; Geographical Variation in Insects. Among the authors are such recognized leaders in their special fields as G. S. Fraenkel, W. M. Hoskins, Robert L. Usinger, W. E. Ripper, and Karl von Frisch, chosen irrespective of country of residence. Each year a different group of subjects will be discussed, though some will merit an annual article, and the authors will vary from year to year. Though supported by abundant references, the articles will not be simply annotated bibliographies. Instead, they will be rea 'able summaries in which recent work will be evaluated by authors qualified to express judgment.

Entomology definitely needs a review publication. No existing work duplicates the Annual Review, which of course will be quite different from an abstract journal such as the Review of Applied Entomology. The number of papers dealing with insects is estimated to be above 4,000 annually, and we all know how difficult it is to keep abreast of progress in specialties, let alone to maintain an adequate general acquaintance with the advances of entomology as a whole. With entomology continually developing wider ramifications and employing more workers in this technological age, we need an annual review for our own use. In Europe news of the forthcoming Annual Review already has brought favorable comment about the value of the publication and the way in which it symbolizes the leadership of American entomologists.

Volume I of the Annual Review is scheduled to appear in February 1956. The publisher is Annual Reviews, Inc., a nonprofit organization with headquarters in Stanford, California. This organization for several years has been publishing annual review volumes in biochemistry, plant physiology, microbiology, and several other sciences. There is every reason to expect that a book on entomology will be as successful as those on comparable scientific fields, though the momentum of the first few volumes may be necessary before financial independence is achieved. One of the outstanding books of our time is the 1952 Yearbook of Agriculture, *Insects*, which has been in great demand, as shown by both sales and free distribution. This book has demonstrated the wide interest in a book containing readable, substantial articles on various aspects of entomology. That it has contributed immensely to the public's understanding of entomological problems is obvious.

Before embarking on the present project, the Board of Directors of Annual Reviews, Inc., sampled opinions of various entomologists and others regarding the venture, and the officers and governing board of the Entomological Society of America were consulted about their willingness to advise on editorial matters and to give financial assistance, if required, during the first four years. Although the judgment of consultants was favorable, Annual Reviews, Inc., would not have gone ahead except for the enthusiasm of individual members of the E.S.A. and the pledged support of the Society as a group. The result was a formal contract which provides for close cooperation between the Society and Annual Reviews, Inc. The Society makes suggestions regarding the selection of members of the editorial board who plan the annual volumes. It also has the opportunity to take pre-publication orders from its members and receive a commission of 10 to 25 per cent, depending on the total number in the consolidated order. Thus for Volume I, priced at $7.00, the Society will receive from 70 cents to $1.75 per copy for books ordered by E.S.A. members through the Society office *before December 15, 1955*. In return, the Society has agreed to reimburse Annual Reviews, Inc., for any deficit after the first year of sales, up to but not to exceed $2,500 for each of the first four volumes. Thereafter the Society will not have this financial responsibility, but will continue to advise and also will receive commissions for prepublication orders from its members. Further details of this agreement were explained by Dr. Steinhaus in his June article.

Admittedly, the financial responsibilities assumed by your officers and governing board are considerable, potentially leading to a cost of up to $10,000 over a 4-year

period. However, these responsibilities were not contracted lightly, but only after considering the need for this publication and the probable benefits to our profession. It is this faith in the response with which entomologists and allied scientists will receive these volumes, together with the hope for the progressive influence of the books on the profession, which is making the Annual Review of Entomology a reality.

How does entomology rate as a profession among other scientists? Entomology in this country does not receive the general recognition that is widely accorded various branches of engineering, nor that enjoyed by certain of the agricultural and biological sciences. Some of us are prone to be sensitive of salary scales for entomologists as compared to those of other workers with comparable training. Within a few minutes one can name ten of our contemporaries, in several fields of entomology, who would distinguish the ranks of the National Academy of Sciences, but how seldom is an entomologist elected to that senior body of scientists. Late in 1954 the President of the Entomological Society of America appointed a Committee on Professional Status and Standards, whose work has carried into 1955. The existence of such a committee shows that a need for improving the status of entomologists has been felt by responsible leaders.

The regard for the profession of entomology held by those outside our ranks is based primarily on accomplishments, especially the way in which the accomplishments are described for others to understand and appreciate. An annual volume summarizing entomology's advances and characterized by readability and clarity, in addition to being thoroughly critical, will do much for entomology. The Annual Review will show entomologists in a favorable light as a thorough-going, cohesive group interested in both basic truths and practical services. It will give you and me an honest pride in our own profession.

Although the Annual Review of Entomology is not being published by the Entomological Society of America, this largest organized group of entomologists in the world, with more than 3,500 members, is the logical group to support the undertaking. Each of us can contribute by ordering a copy of Volume I, thus helping to make the volume financially sound. Then there will be no doubt about the success of future volumes. Remember, this is our profession, and it will be only what we make it.

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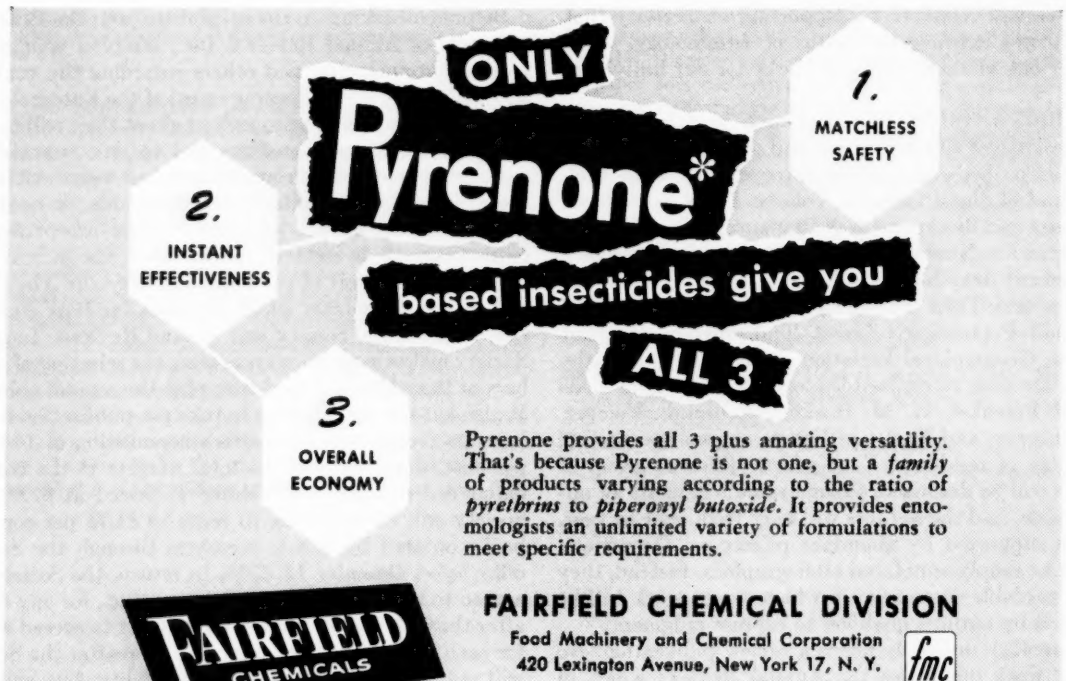
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